Cancer Research

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CTRCAACR
SAN ANTONIO BREAST CANCER SYMPOSIUM

DECEMBER 6-10, 2011
SAN ANTONIO TEXAS USA
WWW.SABCS.ORG

ABSTRACTS

www.aacrjournals.org ACR American Association for Cancer Research
Publishing Information: San Antonio Breast Cancer Symposium

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C. Kent Osborne
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Meeting Profile
For thirty-four years, the mission of the San Antonio Breast Cancer Symposium has been to provide state-of-the-art information on breast cancer research. The symposium aims to achieve a balance of clinical, translational, and basic research, providing a forum for interaction, communication, and education for a broad spectrum of researchers, health professionals, and those with a special interest in breast cancer.

In 2007, the Cancer Therapy & Research Center (CTR) at UT Health Science Center San Antonio and the American Association for Cancer Research (AACR) announced a collaboration for the future of the San Antonio Breast Cancer Symposium. The symposium has been renamed the CTRC-AACR San Antonio Breast Cancer Symposium. Complementing the clinical strengths of the highly regarded annual San Antonio Breast Cancer Symposium, the AACR’s scientific prestige in basic, translational, and clinical cancer research will create a unique and comprehensive scientific meeting that will advance breast cancer research for the benefit of patients.

In 2005, Baylor College of Medicine became a joint sponsor of the symposium and will remain a part of the CTRC-AACR collaboration.

Future Meeting Dates
December 4–8, 2012
December 10–14, 2013
December 9–13, 2014

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2011 Abstracts
34th Annual San Antonio Breast Cancer Symposium
December 6–10, 2011
San Antonio, Texas, USA
www.sabcs.org

Program Schedule

Invited Abstracts

Abstracts

- General Sessions [S1-1 – S6-6] 95s
- Poster Discussion Sessions [PD01-01 – PD10-07] 116s
- Poster Session 1 [P1-01-01 – P1-18-02] 155s
- Poster Session 2 [P2-01-01 – P2-19-04] 245s
- Poster Session 3 [P3-01-01 – P3-18-07] 334s
- Poster Session 4 [P4-01-01 – P4-20-05] 422s
- Poster Session 5 [P5-01-01 – P5-20-07] 512s
- Poster Session Ongoing Trial 1 [OT1-01-01 – OT1-03-03] 599s
- Poster Session Ongoing Trial 2 [OT2-01-01 – OT2-07-02] 608s
- Poster Session Ongoing Trial 3 [OT3-01-01 – OT3-02-04] 619s

Author Index for Abstracts 631s

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DECEMBER 6–10, 2011

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SAN ANTONIO BREAST CANCER SYMPOSIUM

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FUTURE SYMPOSIUM MEETING DATES
35th Annual SABCS  December 4–8, 2012
36th Annual SABCS  December 10–14, 2013
37th Annual SABCS  December 9–13, 2014
**2011 CTRC-AACR
SAN ANTONIO BREAST CANCER SYMPOSIUM
PROGRAM SCHEDULE**

(At press time)
Refer to www.sabcs.org for the most current information.

Room Locations
Exhibit Halls A, B, C & D: Street Level
Ballrooms A & B: Street Level
Bridge Hall: Street Level

**TUESDAY, DECEMBER 6, 2011**

12:00 pm–7:30 pm
REGISTRATION
Bridge Hall
Pre-registered attendees can obtain materials. Those who have not yet registered may do so.

2:00 pm–7:30 pm
EDUCATIONAL SESSIONS
Ballrooms A & B and Exhibit Hall D
Supported by an educational grant from
Susan G. Komen for the Cure®
An update on advances in the technologies available for translational research. Sessions are to provide people with a better understanding of the talks they hear using the techniques described. They also provide researchers with a guide to the techniques they should be considering for their studies.

2:00 pm–3:30 pm
Treatment of Metastatic Breast Cancer – Breast Cancer as a Chronic Disease
Ballroom A
Moderator: Kathleen Pritchard, MD, FRCPC
University of Toronto
Toronto, CANADA

ER positive
Kathleen Pritchard, MD, FRCPC
University of Toronto
Toronto, CANADA

Triple negative breast cancer
Vered Stearns, MD
The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins
Baltimore, MD

HER2 positive
Ian E. Krop, MD, PhD
Dana-Farber Cancer Institute
Boston, MA

Mouse Models of PI3K-Dependent and Basal-Like Breast Cancer
Ballroom B
Moderator: Carlos L. Arteaga, MD
Vanderbilt-Ingram Cancer Center
Vanderbilt University
Nashville, TN

P13K mouse models of breast cancer
Ramon E. Parsons, MD, PhD
Institute for Cancer Genetics
Columbia University
New York, NY

Mouse models of basal-like breast cancer
Jos Jonkers, PhD
Netherlands Cancer Institute
Amsterdam, NETHERLANDS

Patient-derived xenograft models for preclinical breast cancer research: Not just “basal” anymore
Michael T. Lewis, PhD
Baylor College of Medicine
Houston, TX

4:00 pm–5:30 pm
Controversies in Early Breast Cancer
Exhibit Hall D
Moderator: Peter M. Ravdin, MD, PhD
UT Health Science Center San Antonio
San Antonio, TX

Controversies in early breast cancer: Small tumors
Peter M. Ravdin, MD, PhD
UT Health Science Center San Antonio
San Antonio, TX

Chemotherapy benefit in ER positive breast cancer – Mixed messages
Kathy Albain, MD, FACP
Loyola University Chicago Stritch School of Medicine
Maywood, IL

Adjuvant therapy in patients with a borderline HER-2 status
Angelo Di Leo, MD, PhD
Hospital of Prato
Prato, ITALY

New Technologies in Biomarker Discovery for the Clinician
Ballroom A
Moderator: Trey Westbrook, PhD
Baylor College of Medicine
Houston, TX

Metabolomics as a tool to study cancer progression
Arun Sreekumar, PhD
Baylor College of Medicine
Houston, TX

Proteomics
Gordon Mills, MD, PhD
UT MD Anderson Cancer Center
Houston, TX

The potential of epigenetic therapy in breast cancer
Stephen Baylin, MD
Johns Hopkins University
Baltimore, MD

Breast Cancer Trials in Developing Countries - Opportunities and Challenges
Ballroom B
Moderator: Ismail Jatoi, MD, PhD, FACS
UT Health Science Center San Antonio
San Antonio, TX

Challenges in the design and implementation of breast cancer clinical trials in developing countries
Sudeep Gupta, MD, DM
Tata Memorial Hospital
Mumbai, INDIA

Practicality, promise and pitfalls in industry-sponsored trials (in developing countries)
Sandra J. Horning, MD
Genentech, Inc.
South San Francisco, CA
Novel approach to breast cancer in low income countries
David J. Kerr, CBE, MA, MD, DSc FRCP (Glas & Lon), FRCPG (Hon), FMedSci
University of Oxford
Oxford, UNITED KINGDOM

6:00 pm–7:30 pm
Challenges in the Care of Special Populations with Breast Cancer
Exhibit Hall D
Moderator: Mothaffar Rimawi, MD
Baylor College of Medicine
Houston, TX

Oncofertility: Translation in multidimensions
Teresa K. Woodruff, PhD
Northwestern University
Chicago, IL

Treatment of young women with breast cancer
Ann H. Partridge, MD, MPH
Dana-Farber Cancer Institute
Boston, MA

Treatment of older women with breast cancer
Antti Hurria, MD
City of Hope
Duarte, CA

What Makes a Good Target?
Ballroom A
Moderator: Mark D. Pegram, MD
Miller School of Medicine of University of Miami
Miami, FL

Target, identification and drug discovery: What is druggable?
Stephen W. Fesik, PhD
Vanderbilt University Medical Center
Nashville, TN

Clinical validation of new therapeutic targets in breast cancer
Mark D. Pegram, MD
Miller School of Medicine of University of Miami
Miami, FL

Study design considerations in the development of drugs and the diagnostic tests that can guide their use
Steve Shak, MD
Genomic Health, Inc
Hillsborough, CA

Reconstruction
Ballroom B
Moderator: Richard L. Crownover, MD, PhD
Cancer Therapy and Research Center at UT Health Science Center San Antonio
San Antonio, TX

Breast reconstruction with implants and post-mastectomy irradiation: Algorithms and outcomes
Peter G. Cordeiro, MD, FACS
Memorial Sloan-Kettering Cancer Center
New York, NY

Strange bedfellows: Radiation therapy and breast reconstruction
Richard C. Zellars, MD
Johns Hopkins University
Baltimore, MD

Measuring outcomes in breast reconstruction: The patient perspective
Andrea L. Pusic, MD, MHS, FRCS
Memorial Sloan-Kettering Cancer Center
New York, NY

WEDNESDAY, DECEMBER 7, 2011

7:00 am–5:15 pm
REGISTRATION
Bridge Hall

6:45 am–8:15 am
CAREER DEVELOPMENT FORUM: A NETWORKING SESSION FOR YOUNG INVESTIGATORS
Ballroom B
Networking and career development opportunities for early career scientists. The session is open to early-career scientists, defined as graduate students, postdoctoral or clinical fellows, or medical students and residents, who are registered attendees of the SABCS. Space in the workshop is limited to 200 participants; registrations will be accepted on a first-come, first-served basis and is free of charge.

Discussion topics at press time
(Program subject to change)
1. Balancing Research and Clinical Practice
   Ann H. Partridge, Dana-Farber Cancer Institute, Boston, MA
   Judy E. Garber, Dana-Farber Cancer Institute, Boston, MA

2. Balancing Research and Clinical Practice

3. Careers in Industry
   Malte Peters, Novartis, Basel, Switzerland

4. Careers in Industry
   Steven Shak, Genomic Health, Inc., Redwood City, CA

5. Careers in Translational Research
   Rachel Schiff, Baylor College of Medicine, Houston, TX

6. Grant Writing – Clinical/Translational
   Lisa A. Carey, University of North Carolina Lineberger Comprehensive Cancer Center, Chapel Hill, NC

7. Grant Writing – Basic/Translational
   Joan S. Brugge, Harvard Medical School, Boston, MA

8. How to Become a Clinical Trialist
   Hope S. Rugo, UCSF Helen Diller Family Comprehensive Cancer Center, San Francisco, CA

9. How to Get the Most Out of your Fellowship Years – Translational
   Nancy U. Lin, Dana-Farber Cancer Institute, Boston, MA

10. How to Get the Most Out of your Fellowship Years – Clinical
   Rong Li, University of Texas Health Science Center, San Antonio, TX

11. How to Get the Most Out of your Predoctoral Experience
   Valerie M. Weaver, University of California San Francisco Medical Center, San Francisco, CA

12. Leadership Skills
   Carlos L. Arteaga, Vanderbilt-Ingram Cancer Center, Nashville, TN

13. Making the Transition from Fellowship to Faculty
    Pepper Jo Schedin, University of Colorado, Aurora, CO

14. Making the Transition to Independence
    Valerie M. Weaver, University of California San Francisco Medical Center, San Francisco, CA

15. Making the Transition to Independence
    Eric P. Winer, Dana-Farber Cancer Institute, Boston, MA

16. Mentoring and Supervising
    Douglas Yee, University of Minnesota Masonic Cancer Center, Minneapolis, MN

17. Negotiating a Job Offer
    Matthew J. Ellis, Washington University Siteman Cancer Center, St. Louis, MO

Cancer Res; 71(24 Suppl.) December 15, 2011
Cancer Research
December 6-10, 2011

Program Schedule

8:30 am–8:45 am
OPENING REMARKS
Exhibit Hall D

8:45 am–9:15 am
PLENARY LECTURE 1
Exhibit Hall D

Endocrine Therapy for Breast Cancer: Looking Into the Future
Eric P. Winer, MD
Dana-Farber Cancer Institute
Boston, MA

9:15 am–11:30 am
GENERAL SESSION 1
Exhibit Hall D

Moderator: Kathy D. Miller, MD
Indiana University School of Medicine
Indianapolis, IN

9:15
S1-1. A Phase III randomized trial of anastrozole versus tamoxifen in postmenopausal women with metastatic breast cancer: SWOG S0226
Mehta RS, Barlow WE, Albain KS, Vandenberg T, Dakhil SR, Tirumali NR, Lew DL, Hayes DF, Gralow JR, Livingston RB, Hortobagyi GN. University of California at Irvine; Chao Family CCC, Orange, CA; SWOG, Seattle, WA; Loyola University Chicago Stritch School of Medicine, Maywood, IL; London Regional Cancer Program, London, ON, Canada; Wichita CCOP, Wichita, KS, NW Permanente, Portland, OR, University of Michigan, Michigan; Seattle Cancer Care Alliance, Seattle, WA; Arizona Cancer Center, Tucson, AZ; University of Texas/MD Anderson Cancer Center, Houston, TX.

9:30
S1-2. Long-term follow-up in ABCSG-12: Significantly improved overall survival with adjuvant zoledronic acid in premenopausal patients with endocrine-receptor–positive early breast cancer
Gnant M, Milinker B, Luschin-Ebgren G, Stoeger H, Dubsky P, Jakesz R, Singer C, Eidtmann H, Fesl C, Eiermann W, Marth C, Greil R. Medical University of Vienna, Vienna, Austria; Paracelsus Medical University Salzburg, Salzburg, Austria, Medical University of Graz, Graz, Austria; University of Schleswig-Holstein, Kiel, Germany; Austrian Breast and Colorectal Cancer Study Group, Vienna, Austria; Red Cross Women’s Hospital, Munich, Munich, Germany; Medical University of Innsbruck, Innsbruck, Austria.

9:45
S1-3. Long-term survival outcomes among postmenopausal women with hormone receptor-positive early breast cancer receiving adjuvant letrozole and zoledronic acid: 5-year follow-up of ZO-FAST
de Boer R, Bundner N, Eidtmann H, Neven P, von Minckwitz G, Martin N, Modi A, Coleman R. Royal Melbourne Hospital, Victoria, Australia; University Hospital of South Manchester NHS Foundation Trust, Manchester, United Kingdom; University Frauenklinik, Kiel, Germany; UZ Gasthuisberg, Leuven, Belgium; German Breast Group, Frankfurt, Germany; Novartis Oncology, East Hanover, NJ, University of Sheffield, Sheffield, United Kingdom.

10:00
Discussion
James N. Ingle, MD, Mayo Clinic College of Medicine, Rochester, MN

10:15
S1-4. Retrospective analysis of study EGF30008 by mass-spectrometry based serum assay (VeriStrat®)

10:30
S1-5. Modulation of cancer and stem cell biomarkers by the Notch inhibitor MK-0752 added to endocrine therapy for early stage ER+ breast cancer

10:45
S1-6. Characterization of breast cancer distant metastasis based on outcome over time using a gene expression profiling approach and identification of pathway activities of late relapse

11:00
S1-7. Molecular tumor characteristics influence adjuvant endocrine treatment outcome
Bianchini G, PuzzaI L, Ismaiel T, Kelly CM, Zambetti M, Caralo A, Del Conte G, Santapia L, Symmans WF, Gianni L. San Raffaele Comprehensive Cancer Center, Milan, Italy; University of Texas, MD Anderson Cancer Center, Houston, TX; Mater Misericordiae University Hospital, Dublin, Ireland; Hospital of Prato and Istituto Toscana Tumori, Prato, Italy.

11:15
S1-8. Molecular signaling distinguishes early ER positive breast cancer recurrences despite adjuvant tamoxifen

11:30 am–12:00 pm
AACR DISTINGUISHED LECTURESHIP IN BREAST CANCER RESEARCH
Exhibit Hall D

Protective Adaptive Responses to Breast Cancer Therapies
Joan S. Brugge, PhD
Harvard Medical School
Boston, MA

12:00 pm–1:35 pm
LUNCH

12:15 pm–1:15 pm
PRODUCT THEATRE
Exhibit Hall C – Exhibit Area

Updates in Breast Cancer in Diagnostic Testing
Sponsored by Clement, A GE Healthcare Company

12:30 pm–1:35 pm
CLINICAL SCIENCE FORUM
Medical Economics - The Cost of Care Ballroom A

Moderator: Eric P. Winer, MD
Dana-Farber Cancer Institute
Boston, MA

The cost of cancer care: How much do we spend and how can we spend it better?
Bena Elkin, PhD
Memorial Sloan-Kettering Cancer Center
New York, NY

Health care reform and cost control: Practical and ethical considerations for cancer care providers
Michael Hassett, MD, MPH
Dana-Farber Cancer Institute
Boston, MA

www.aacrjournals.org
2:30  S2-2. Luminal A subtype predicts radiation response in patients with T1N0 breast cancer enrolled in a randomized trial of tamoxifen with or without breast radiation

2:45  S2-3. NSABP protocol B-34: A clinical trial comparing adjuvant clodronate vs. placebo in early stage breast cancer patients receiving systemic chemotherapy and/or tamoxifen or no therapy — final analysis
Paterson AHG, Anderson SJ, Lembersky BC, Felehrenbacher L, Falkson CI, King KM, Weir LM, Brufsky AM, Dalhui S, Lad T, Baez-Diaz L, Gralow JR, Robidoux A, Perez EA, Zheng P, Geyer CE, Swain SM, Costantino JP, Mamounas EP, Wolmark N. National Surgical Adjuvant Breast and Bowel Project (NSABP), Tom Baker Cancer Centre; University of Pittsburgh Graduate School of Public Health; University of Pittsburgh Cancer Institute School of Medicine; Kaiser Permanente, Northern California; University of Alabama at Birmingham/ECOG; Cross Cancer Institute; British Columbia Cancer Agency; University of PittsburghMagee Women’s Hospital; Cancer Center of Kansas; Stroger Hospital Cook County MBCCP; San Juan MBCCP; University of Washington/SWOG; Centre Hospitalier de l’Université de Montréal; Mayo Clinic Jacksonville/NCCTG; Allegheny General Hospital; Washington Cancer Institute, Washington Hospital Center; Aultman Health Foundation.

3:00  S2-4. GAIN (German Adjuvant Intergroup Node Positive) study: A phase-III multicenter trial to compare dose dense, dose intense ETC (iddETC) vs. EC-TX and Ibandronate vs. observation in patients with node-positive primary breast cancer – 1st interim efficacy analysis

3:15  S2-5. An anti-HER3 antibody that stabilizes the inactive conformation inhibits both HER2 and ligand driven tumor growth
Gamer AP, Bialucha CU, Chen D, Elis W, Kunz C, Li S, Munier J, Saxena P, Sineshchekova O, Sprague E, Ettenberg S. Novartis Institutes for Biomedical Research, Cambridge, MA; Morphosys AG, Munich, Germany; Sanofi-Aventis, Cambridge, MA.

3:30  S2-6. ErbB3 expression is required for maintenance of normal and transformed luminal breast epithelial cells
Cook RS, Soko JM, Rineshart C, Miller TW, Poljak K, Prat A, Perou CM, Arteaga CL. Vanderbilt University, UNC Lineberger Comprehensive Cancer Center, Dana Farber Cancer Institute.

3:45  S2-7 Mechanisms of action and biological significance of HER2 mutations in HER2 overexpressing breast cancer
Boulbes DR, Jin Q, Arold ST, Ladbury JE, Yu D, Estaeva FJ. University of Texas – MD Anderson Cancer Center, Houston, TX; Mynad Genetics, Salt Lake City, UT.

4:00 pm–5:00 pm  SUSAN G. KOMEN FOR THE CURE® BRINKER AWARDS FOR SCIENTIFIC DISTINCTION LECTURES
Exhibit Hall D
The Basic Science award is presented to a researcher who has added substantively to our understanding of the basic biology of, or development of, methodologies that further our ability to unravel the genetic and molecular basis of breast cancer. This year the award is being presented to:...
P1-01-01  A Rat Monoclonal Antibody Against Bone Sialoprotein II Is Active in Preventing and Treating Tumor Growth and Osteolytic Lesions in Nude Rats Induced by MDA-MB-231 Breast Cancer Cells
Berger MR, Zepp M, Armburster FP. DKFZ, Heidelberg, Germany; Immunodiagnostics Comp, Bensheim, Germany.

P1-01-02  T Cell Is a Key Player in the Establishment of Cancer Associated Pre-Metastatic Bone Disease

P1-01-03  The Cytotoxicity of Select Neutrophils in Cancer Patients and the Role of Chemokines in Inducing Neutrophil Cytotoxicity
Comer E, Granot Z, Norton L, Beneza R. Memorial Sloan-Kettering Cancer Center, New York, NY.

P1-01-04  Immunological Effects of Bisphosphonates on γδT Cells in Breast Cancer
Sugie T, Tanaka Y, Toi M, Minato N. Graduate School of Medicine, Kyoto University, Kyoto, Japan.

P1-01-05  Conditioning by the Tumor Environment Turns Invariant Natural Killer T Cells into Negative Regulators of Anti-Tumor Immunity Elicited by Treatment
Pilones KA, Demaria S. NYU School of Medicine, New York, NY.

P1-01-06  Mechanisms of Tumor Immune Escape in Triple-negative Breast Cancers (TNBC) with and without Mutated BRCA1
Segerer SE, Kapp M, Hahne JC, Dietl J, Engel JB. Medical University of Vienna, Wurzburg, Wurzburg, Germany.

P1-01-07  ErbB-2 Peptide Vaccination Suppresses Spontaneous Tumorigenesis and Tumor Stem Cell Expansion in MMTV-PyVT Transgenic Mouse
Park KH, Gil EY, Choi YJ, Kim ST, Cho K, Seo JH, Lee ES, Kim IS, Disis ML. Korea University College of Medicine, Seoul, Korea; Korea University College of Medicine; University of Washington, Seattle, WA.

P1-01-08  Expression of Interleukin-15 (IL-15) and the IL-15 Receptor in Human Breast Cancer
Sanders AJ, Ye L, Wei XQ, Marsel RE, Jiang WY. Cardiff University School of Medicine, Cardiff, Wales, United Kingdom; Cardiff University, Cardiff, Wales, United Kingdom.

P1-01-09  A Focused Immune Response Targeting the Homotypic Binding Domain of the Carcinoembryonic Antigen Blocks the Establishment of Tumor Foci in Vivo
Gatgey J, Abdul-Wahid A. University of Toronto, Toronto, ON, Canada; Sunnybrook Research Institute, Toronto, ON, Canada.

P1-01-10  Immune Suppression of Regulatory T Cells and M2 Macrophage in Breast Cancer Patients
Imoto S, Sakamura N, Kamma H, Nakatsuura T. School of Medicine, Kyorin University, Mitaka, Japan; Research Center Organization, National Cancer Center Hospital East, Kashiwa, Japan.

P1-01-11  CD4+CD25+CD127+ Regulatory T Cells Have Immunosuppressive Function in Patients with Breast Cancer
Sakamura N, Nakatsumawa M, Ito H, Isaka H, Imai K, Tazaki E, Miyamoto K, Wada N, Imoto S, Nakatsuura T. Graduate School of Medicine, Kyorin University, Mitaka Tokyo, Japan; National Cancer Research Center Hospital East, Kashiwa Chiba, Japan.

P1-01-12  Mesoporous Silicon Particles for the Presentation of Tumor Antigens and Adjuvant for Anti-Cancer Immunity
Menzel IM, Melendez B, Gu J, Serda RE. Methodist Hospital Research Institute, Houston, TX.

P1-01-13  Prognostic Impact of CD8 in Node-Negative Breast Cancer
Schmidt M, Chen Z, Hellwig B, Böhm D, Liebrecht A, Gebhard S, Gehrmann M, Koebl H, Hengstler JG. University Hospital, Mainz, Germany; Technical University, Dortmund, Germany; Bayer GMBH, Leverkusen, Germany.

P1-01-14  Gene Expression of Immune Mediators within Nipple Aspirate Fluid and Ductal Lavage from Normal and Cancerous Breasts
Love SM, Ruffell B, Nguyen T, Mills D, Coussens LM. Dr. Susan Love Research Foundation, Santa Monica, CA; University of California-San Francisco, San Francisco, CA.

P1-01-15  Do Serum Cytokines Predict Breast Cancer Behavior?
Lush E, Dedert E, Daup M, Dhabhar F, Spiegel D, Tille J, Mccmasters K, Sephton SE, Chappar A. University of Louisville; Duke University Medical Center, Veterans Affairs Medical Center, Stanford University School of Medicine; James Graham Brown Cancer Center, University of Louisville School of Medicine, Yale School of Medicine.

P1-01-16  Detecting a Breast Carcinoma-Deriving B-Cell Response: An Immunoproteomics Biomarker Approach
Keller K, Boehm DJ, Grus FH, Koebl H. University Medical Center Mainz, Germany.

Tumor Cell Biology: Epithelial-Mesenchymal Transition

P1-02-01  c-Jun N-Terminal Kinase 1 (JNK1) Inhibits Tumor Growth and Metastasis by Downregulating Epithelial to Mesenchymal Transition (EMT) and Stem Cell-Related Genes
Ebelt ND, Van Den Berg CL. The University of Texas, Austin, TX.

P1-02-02  Zoledronic Acid Reverses the Epithelial-Mesenchymal Transition While Inhibiting the Tumor Initiating Cell Population of Highly Tumorigenic Breast Cancer Cell Lines
Schech AJ, Gilani RA, Kazi AA, Brodie JH. University of Maryland, Baltimore, Baltimore, MD.

P1-02-03  The Reciprocal Roles of E-Cadherin and ZEB1 Demonstrate the Mesenchymal-Epithelial Transition as a Primary Characteristic of Inflammatory Breast Cancer
Chu K, Boley KM, Luo AZ, Ye Z, Wright MC, Freiter EM, Robertson FM. The University of Texas MD Anderson Cancer Center, Houston, TX.

P1-02-04  Estrogen Receptor beta Inhibits Breast Cancer EMT by Regulating the Expression of miR-200
Invasive Lobular Carcinoma - A Luminal Breast Cancer
Histotype Enriched for Epithelial-to-Mesenchymal Transition Features

P1-02-06
Silencing of IGF-1R Has Paradoxical Effects in Triple Negative Breast Cancer Phenotypes
Talaiferro-Smith LD, Oberlick EM, Liu T, Eggers C, Kline ER, Nagaraju GP, Marcus A, O'Regan R. Winship Cancer Institute at Emory University, Atlanta, GA; Grady Memorial Hospital, Atlanta, GA.

Epithelial-Mesenchymal Transition Correlated with Serum Cytokine Profiling in Breast Cancer Patients
Giordano A, Cohen EN, Anfossi S, Gao H, Lee B-N, Mego M, Sandra T, Valero V, Alvarez RH, Cristofanilli M, De Placido S, Hertogaboy GN, Woodward W, Ueno NT, Reuben JM. The University of Texas MD Anderson Cancer Center, Houston, TX; University Federico II of Naples, Naples, Italy; Fox Chase Cancer Center, Philadelphia, PA; National Cancer Institute, Bratislava, Slovakia (Slovak Republic).

Modulation of EMT by Targeting E-Cadherin Restores Radiation Sensitivity in Human Breast Cancer Cells
Munshi A, Yuan Y, Liu J, Meyn RE. University of Oklahoma Health Sciences Center, Oklahoma City, OK; Baylor College of Medicine, Houston, TX; The University of Texas M.D. Anderson Cancer Center, Houston, TX.

Epigenetics-Regulated microRNAs Related with Epithelial-Mesenchymal Transition of Breast Cancer Cells
Sato F, Ito T, Tsuchiya S, Kawaguchi-Sakita N, Shimizu K, Tsujimoto G, Toi M. Graduate School of Medicine, Kyoto University, Kyoto, Japan; Graduate School of Pharmaceutical Sciences, Kyoto University, Kyoto, Japan.

Vimentin Expression: as a Prognostic Factor and a Possible Molecular Target of Triple Negative Breast Cancer

The BCL2 Antagonist of Death, baD is Down-regulated in Fibroadenomatoid Changes with a higher occurrence rate in Middle-aged benign breast disease patients with the trend retained in cancer Patients
Munshi A, Yuan Y, Liu J, Meyn RE. University of Oklahoma Health Sciences Center, Oklahoma City, OK; Baylor College of Medicine, Houston, TX; The University of Texas M.D. Anderson Cancer Center, Houston, TX.

The p120ctn is a key effector of Ras-PKCε-mediated oncogetic signaling

EBCM Stiffness and Breast Tumor Histology and Treatment Phenotype
Acerbi I, Au C, Chen Y-Y, Park C, Huang S, Weaver V. Center for Bioengineering and Tissue Regeneration, University of California, San Francisco, San Francisco, CA; Carol Buck Breast Cancer Center, University of California San Francisco, San Francisco, CA; University of California San Francisco, San Francisco, CA; Institute of Regeneration Medicine, University of California San Francisco, San Francisco, CA; University of California at San Francisco, San Francisco, CA.

The Norton-Simon Hypothesis and Cancer Stem Cells: How Cancer Stem Cells May Explain the Effectiveness of Dose-Dense Chemotherapy
Landis MD, Dobrolecki LE, Wong H, Lai Q, Vahdat LT, Chang JC. The Methodist Hospital Research Institute, Houston, TX; Weill Cornell Medical College, New York, NY.

Adaptive Exploitation of Stromal Cell Metabolism by Tumor Cells

In Vitro Characterization of a Breast Cancer Microenvironment with Epithelial-to-Mesenchymal Transition (EMT) Characteristics
Casbas-Hernandez P, Roman-Perez E, Mani SA, Treoester MA. School of Medicine, UNC, Chapel Hill, NC; Gillings School of Public Health, UNC, Chapel Hill, NC; UNC Hospitals, Chapel Hill, NC; University of Texas MD Anderson Cancer Center, Houston, TX.

Fibrocytic Changes Have Different Age-Dependent Patterns in Benign, In S itu, and Invasive Breast Cancer Patients
Bekhash A, Kovatich AJ, Chen Y, Hooke JA, Kvecher L, Mural RJ, Shriver CD, Hu H. Windber Research Institute, Windber, PA; Walter Reed Army Medical Center, Washington, DC; MDR Global Systems LLC, Windber, PA.

Fibroblastic Changes Have a Higher Occurrence Rate in Middle-Aged Benign Breast Disease Patients with the Trend Retained in Breast Cancer Patients
Bekhash A, Hooke JA, Chen Y, Kovatich AJ, Kvecher L, Mural RJ, Shriver CD, Hu H. Windber Research Institute, Windber, PA; Walter Reed Medical Center, Washington, DC; MDR Global Systems LLC, Windber, PA.

Subtype-Specific Gene Expression Signatures in Peritumoral Non-Neoplastic Breast Tissue
Williams T, Roman-Perez E, Rein JL, Amos KD, Troester MA. University of North Carolina-Chapel Hill, Chapel Hill, NC.

Adipose Tissue in Breast Cancer: Not an Idle Bystander but an Active Participant in Breast Cancer Progression

Significance of FAP, SMA and CD31 Expression in the Strom a of Breast Cancer

S100a7 enhances breast tumor progression and metastasis through activation of pro-metastatic and inflammatory pathways
Ganjura RK, Nasser MW, Qamri Z, Deol YS, Schwendener RA, Leone G, Wolf R, Yuspa S. The Ohio State University Medical Center, Columbus, OH; University of Zurich, Zurich, Switzerland; Ludwig Maximilian University, Munich, Germany; National Institutes of Health, Bethesda, MD.

The Mechanism of Anti-Breast Cancer TICs Effect of Pyrvinium Pamoate Is through WNT/beta-Catenin Signaling

In Vitro Qualification and Quantification of murine mammary tumor Cell biology: Stem/progenitor Cells
p1-03-01
p1-04-01
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p1-03-06
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p1-01-07
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P1-04-03  The Effect of Survivin Downregulation on Radiosensitization of Breast Cancer Cell Lines Grown under Adherent and Stem Cell Promoting Culture Conditions
Debeb BG, Larson R, Xu W, Lacerda L, Reuben JM, Buchholz TA, Ueno NT, Woodward WA. Morgan Welch witchford Breast Cancer Clinic and Research Group, The University of Texas M.D. Anderson Cancer Center, Houston, TX.

P1-04-04  Focal Adhesion Kinase Plays a Major Role in the Regulation of Human Ductal Carcinoma In Situ (DCIS) Stem Cell Activity
Williams KE, Bundjed NJ, Farnie G. University of Manchester, Paterson Institute for Cancer Research, Manchester, United Kingdom; University Hospital of South Manchester, Manchester, United Kingdom.

Honig A, Diessner J, Dietl J, Wischhusen J. University of Wuerzburg, Wuerzburg, Germany.

P1-04-06  Ionizing Radiation Reprograms Non-Tumorigenic Cancer Cells into Cancer Stem Cells
Chann L, Vlasi E, Lorenza DD, Dekremzan C, Pajonk F. University of California, Los Angeles, CA.

P1-04-07  Poly (ADP-Ribose) Polymerase-1 (PARP-1) Is Overexpressed in Human Breast Cancer Stem Cells: Results from a Proteomic-Based Approach
Gilbert M, Ginestet C, Audebert S, Pophillat M, Torion Y, Birnbaum D, Borg J-P, Charafe-Jauffret E, Goncalves A. Institut Paoli-Calmettes, Marseille, France; U91 INSERM, Marseille, France; Université de la Méditerranée, Marseille, France; Centre d’Immunologie Marseille-Luminy, Marseille, France.

P1-04-08  Distribution of ALDH1 Positive Stem Cells in Benign Mammary Tissue from Women with and without Breast Cancer
Iffoss BL, Holmqvist B, Alm P, Olsson H. Telemark Hospital, Sken, Lund University, Lund, Sweden.

P1-04-09  Biphasic Effects of Docetaxel and Hedgehog Signaling Antagonists on Breast Cancer Tumor-Initiating Cells In Vivo
Zhang X, Moraes RC, Landis MW, Wu M-F, Hilsenbeck SG, Cairo MM, Tofigar R, Chang JC, Lewis MT. Baylor College of Medicine, Houston, TX; Karolinska Institutet, Novum, Sweden.

Tumor Cell Biology: Epigenetics

P1-05-01  PELP1 Oncogenic Functions Involve Regulation of Histone Arginine Methylation
Mann M, Cortez V, Yang BW, Telma RR, Vadlamudi RK. University of Texas Health Science Center at San Antonio, San Antonio, TX; University of Southern California, Los Angeles, CA.

P1-05-02  Epigenetic Regulation by Alcohol Reactivates Estrogen Receptor alpha in Estrogen Receptor alpha-Negative Cells
Wong AW, Nunez N. University of Texas at Austin, Austin, TX.

P1-05-03  Relationship between Polycomb Repressive Complex EZH2/CBX7, Large Non-Coding RNA ANRIL and Stem Cells Biomarkers in Triple Negative Breast Carcinomas: Important Role in Carcinogenesis through an Epigenetic Silencing Process and New Clues for Targeted Therapies

P1-05-04  Distinct Patterns of Promoter CpG Island Methylation of Breast Cancer Subtype Are Associated with Different Stem Cell Phenotype
Park SY, Kwon HJ, Choi Y, Lee HE, Kim S-W, Kim JH; Kim IA, Jung N, Cho N-Y, Kang GH. Seoul National University Bundang Hospital; Seoul National University Hospital; Cancer Research Institute, Seoul National University.

P1-05-05  Prognostic Utility of Histone Modifier Gene Expression Profiles in Human Breast Cancer
Patani N, Jiang WG, Newbold RF, Mobbel K. The London Breast Institute, The Princess Grace Hospital, London, United Kingdom; University Department of Surgery, Cardiff University School of Medicine, Cardiff, United Kingdom; Brunel Institute of Cancer Genetics and Pharmacogenomics, Brunel University, London, United Kingdom.

P1-05-06  Hypermethylation 14-3-3-Sigma Promoter as a Biomarker to Screening for Metastasis and Potential Prognostic Factor in Breast Cancer Patients
Martinez-Galan J, Torres-Torres B, Gonzalez-Vicente A, Ruiz-Vozmediano J, Delgado-Perez JR. Hospital Universitario Virgen de las Nieves, Granada, Spain; Centro de Investigaciones Biomédicas, Granada, Spain.

Prognosis/Response Predictions: Response Predictions – I
P1-06-01  Withdrawn

P1-06-02  Correlation between Gene Variants in CYP19 (Aromatase) and TCL1A with Disease and Tolerability Endpoints in the ATAC Trial
Ray C, Regnier C, Lidereau R, Bieche I. Institut Curie Hôpital Rene Huguenin, St Cloud, France.

P1-06-03  Predictive Value of HER2, Topoisomerase-IIa (Topo-II) and Tissue Inhibitor of Metalloproteinases (TIMP-1) for Efficacy of Taxane-Based Chemotherapy in Intermediate Risk Breast Cancer - Results from the EC-Doc Trial
Gluz O, Erber R, Kates R, Kreipe H, Barlet A, Liedtke C, Pelz E, Hueber J, Kuhn W, Nitz U, Hartmann A, Harbeck N, Brunner N. West German Study Group, Muenchegendabach, Germany; University Hospital Erlangen, Erlangen, Germany; Medizinische Hochschule Hannover, Hannover, Germany; University Copenhagen, Copenhagen, Denmark; University Hospital Muenster, Muenster, Germany; Pathology Practice, Viersen, Germany; University Hospital Tuebingen, Tuebingen, Germany; Kantonsspital St. Gallen, St. Gallen, Switzerland; University Hospital Bonn, Bonn, Germany; University Hospital Cologne, Cologne, Germany.

P1-06-04  PAM50 Prognostic Index Predicts Response to Weekly Adjuvant Paclitaxel in Node-Positive Operable Breast Cancer
Martin M, Rodriguez-Lescure A, Stijlemans U, Munárriz B, Ruiz-Borrego M, Davis C, Crespo C, Rodríguez CA, Ebbert MTW, Alvarez I, Furido V, Bastien RRL, Garcia AM, Cheung MC, Palacios J, Ellis MJC, Carrasco E, Casas MI, Caballero R, Perez CM, Bernard PS. Hospital General Universitario Gregorio Marañon, Madrid, Spain; Hospital Universitario de Elche, Elche, Spain; University of Utah Health Sciences Center/ Huntsman Cancer Institute, Salt Lake City, UT; Hospital Universitario La Fe, Valencia, Spain; Hospital Universitario Virgen del Rocio, Sevilla, Spain; Hospital Universitario Ramon y Cajal, Madrid, Spain; Hospital Clinico Universitario de Salamanca, Salamanca, Spain, The ARLP Institute for Clinical and Experimental Pathology, Salt Lake City, UT; Hospital de Donostia, San Sebastian, Spain; Hospital Universitario San Carlos, Madrid, Spain; University of North Carolina at Chapel Hill; Washington University in St Louis, St Louis, Spain; Spanish Breast Cancer Research Group, GECAM, San Sebastian de los Reyes, Madrid, Spain.

P1-06-05  Withdrawn

P1-06-06  The Importance of CCK10 and CCKR3-A in Breast Cancer
Hilborn E, Sivik T, Kot A, Fomander T, Skoog L, Nordensjöld B, Stål O, Jansson A. Faculty of Health Sciences, Linköping, Sweden; Karolinska Institutet.
P1-06-07  TIMP-1 in Combination with HER2 and TOP2A for Prediction of Benefit from Adjuvant Anthracyclines in High Risk Breast Cancer Patients
Hertel PB, Tu D, Ejlertsen B, Jensen M-B, Bagduse E, Jiang S, O’Malley FP, Pritchard KJ, Shepherd LE, Bartels A, Brünnner N, Nielsen TO, Faculty of Life Sciences, Univ of Copenhagen, Copenhagen, Denmark; National Cancer Institute of Canada, Kingston, ON, Canada; Rigshospitalet, Copenhagen, Denmark; Danish Breast Cancer Cooperative Group, Copenhagen, Denmark; Herlev Hospital, Herlev, Copenhagen, Denmark; Mount Sinai Hospital, Toronto, ON, Canada; University of Toronto, Toronto, ON, Canada; University of British Columbia Vancouver, Vancouver, BC, Canada.

P1-06-08  Effect of Treatment Emergent Symptoms on Relapse Free Survival: NCIC CTG MA.12 a Randomized Placebo-Controlled Trial of Tamoxifen after Adjuvant Chemotherapy in Pre-Menopausal Women in Early Breast Cancer
Chapman J-AW, Shepherd LE, Le Maitre A, Pritchard KJ, Graham BC, Gelmon KA, Bramwell VH. NCIC Clinical Trials Group, Queen’s University, Kingston, ON, Canada; University of Toronto, ON, Canada; University of British Columbia Vancouver, Vancouver, BC, Canada; Tom Baker Cancer Centre, Calgary, AB, Canada.

P1-06-09  Patient-Specific Integrative Pathway Analysis Using PARADIGM Identifies Key Activities in I-SPY 1 Breast Cancer Patients (CALGB 150007/150012; ACRIN 6657)

P1-06-10  Lobular Breast Cancer and NAC: Combined Results from the NKI and I-SPY 1 Trial

P1-06-11  Comparison of Community and Central Her2 Assessment on Outcome of Neoadjuvant Chemotherapy in the I-SPY 1 Trial
DeMichele A, Yau C, Zhu J, Wu H, Kuhle J, Lomburg M, Buxton M, Davis S, Mies C, Livasy C, Chin K, Gray J, Carey L, Esserman L, Petricoin E. Abramson Cancer Center, University of Pennsylvania, Philadelphia, PA; University of California, San Francisco, CA; George Mason University, Fairfax, VA; University of California, Santa Cruz, CA; Carolina HealthCare System, Charlotte, NC; Oregon Health Sciences University, Portland, OR; The University of North Carolina at Chapel Hill, Chapel Hill, NC; University of Pennsylvania, Philadelphia, PA.

P1-06-12  Circulating Tumor Cells (CTC) Monitoring during Phase II Study with Lapatinib (L) and Capecitabine (C) in Patients with Brain Metastases from HER2-Positive (+) Metastatic Breast Cancer (MBC) before Whole Brain Radiotherapy (WBR): LANDSCAPE Study

P1-06-13  An Amplicon-Driven Aromatase Inhibitor Response (ADAIR) Signature Provides an Orthogonal Risk Classifier for ER+ Breast Cancer

P1-06-14  Topoisomerase II alpha (Top2a) Protein Expression Is a Predictor for Response to Anthracycline-Based Chemotherapy (ATC-CT): Is It Due to Gene Amplification, HER2-Coamplification or a Summation of Pathways Leading to This Highly Proliferative Phenotype?
Abdel-Fatah TMA, Lambros MB, Vatcheva R, Ball G, Dickinson PD, Moseley P, Green AR, Ellis IO, Reis-Filho JS, Chan S. Nottingham University City Hospital, NHS Trust, Nottingham, Nottinghamshire, United Kingdom; The Institute of Cancer Research, London, United Kingdom; School of Molecular Medical Science, Nottingham University, Nottingham; Nottingham Trent University, Nottingham.

P1-06-15  A Genomic Predictor Developed from Breast Cancer Cell Line Pre-Survs Both Disease-Free Survival and Overall Survival in Breast Cancer Patients Treated with Doxorubicin and Cyclophosphamide: A Collaborative Project of the NSABP and Precision Therapeutics

P1-06-16  BRCA2 Mutation Carriers Respond Poorly to Conventional Anthracylins/Taxanes-Based Neo-Adjuvant Chemotherapy

P1-06-17  A New Pathological Response Index (PRI) for Neoadjuvant Chemotherapy Accurately Predicts Clinical Outcomes of Locally Advanced Breast Cancers (LAPBC)
Abde-Fatah TMA, Moseley P, Lee A, Balls G, Ellis IO, Chan S. Nottingham University City Hospital, Nottingham, Nottinghamshire, United Kingdom; Nottingham University, Nottingham, United Kingdom; The Institute of Cancer Research, London, Nottingham Trent University.

P1-06-18  Loss of E-Cadherin Expression in Clinical Breast Cancer Is Associated with an Adverse Outcome on Tamoxifen
Hiscox S, Rahka E, Smith C, Farnow L, Gandahara S, Green A, Ellis I, Barrett-Lee P, Nicholson RI, Gee J. Cardiff University, Cardiff, Wales, United Kingdom; Nottingham University Hospital, Nottingham, United Kingdom; Velindre Hospital, Cardiff, Wales, United Kingdom.

P1-06-19  Absence or Low Levels of c-Jun NH2-Terminal Protein Kinase (JNK) Mitogen Activated Protein Kinase (MAPK) Is Related to a Luminal B Subtype and an Impaired Survival in Patients with an ER Positive Breast Cancer Treated with Adjuvant Tamoxifen
Linderholm BK, Sanchez-Chavez B, Hellborg H, Johansson UM, Leuwensroh R, Viktorsson K, Kanter L. Institution of Oncology, Sahlgrenska University Hospital, Gothenburg, Sweden; Institution of Oncology and Pathology, Karolinska Institute, Stockholm, Sweden; Institution of Pathology, Herlev, Denmark.

P1-06-20  Response to Neo-Adjuvant Chemotherapy and Outcomes for I-SPY 1 Patients Stratified by the 70-Gene Prognosis Signature (MammaPrint) and Molecular Subtyping (Blueprint)
Gluck S, de Snoo F, Tian S, Glas A, van’t Veer L. Miller School of Medicine, University of Miami, Miami, FL; Agenda, Amsterdam, Netherlands; HDFC Cancer Center, UCSI, San Francisco, CA.
P1-06-21 Relationship between Body Mass Index and Preoperative Treatment Response to Aromatase Inhibitor Exemestane in Postmenopausal Patients with Primary Breast Cancer

Takada M, Saji S, Masuda N, Kuroi K, Sato N, Takei H, Yamamoto Y, Ohno S, Yamashita H, Hisamatsu K, Aogi K, Iwata H, Ueno T, Sasano H, Toi M. Graduate School of Medicine, Kyoto University, Japan; Saitama Medical University, International Medical Center, Japan; Osaka National Hospital, Japan; Tokyo Metropolitan Cancer and Infectious Diseases Center, Komagome Hospital, Japan; Niigata Cancer Center Hospital, Japan; Saitama Cancer Center, Japan; Kumamoto University, Japan; National Kyushu Cancer Center, Japan; Nagaoy City University Hospital, Japan; Hiroshima City Asa Hospital, Japan; National Shikoku Cancer Center, Japan; Aichi Cancer Center Hospital, Japan; Tohoku University Hospital and School of Medicine, Japan.

P1-06-22 Identification of Biomarkers in Breast Cancer for Prediction of Response to PARP Inhibitor Olaparib


P1-06-23 Changes in Gene Expression after One Dose of Trastuzumab (T) in HER2+ Breast Cancer Cell Lines Predict Novel Pathways of Response in HER2 Positive Early Stage Breast Cancer

Sprecher E, Lezon-Geya K, Sarkar S, Bossuyt V, Narayaan M, Kumar A, Krop I, Winer E, Tuck D, Kleinstein S, Harris L. Yale University, New Haven, CT; Yale University; Beckman Coulter, Stony Brook University Hospital, Stony Brook, NY; Dana-Farber Cancer Institute, Boston, MA.

P1-06-24 Nuclear Localization of Stat5a predicts Response to Antiestrogen Therapy and Prognosis of Clinical Breast Cancer Outcome

Peck AR, Witkiewicz AK, Liu C, Klimowicz AC, Stringer GA, Pequignot E, Freydin B, Yang N, Tran TH, Rosenberg AL, Hooke JA, Kovatch AJ, Shriver CD, Rimm DL, Magliocco AM, Hyslop T, Rui H. Thomas Jefferson University, Philadelphia, PA; Tom Baker Cancer Center, Calgary, AB, Canada; Walter Reed Army Medical Center, Washington, DC, MDR Global Systems, LLC, Windber, PA; Yale University School of Medicine, New Haven, CT.

P1-06-25 Changes in FDG PET SUV Correlates with Ki-67 Following 2 Weeks of Aromatase Inhibitor Therapy in ER+ Early Stage Breast Cancer, a Pilot Imaging Study

Gadi VK, Kurland BF, Specht JM, Rodller E, Korde LA, Peterson LM, Schubert EK, Chai X, Mankoff DA, Lindem HM. University of Washington, Seattle, WA; Fred Hutchinson Cancer Research Center, Seattle, WA.

P1-06-26 The EndoPredict Score Is a Response Predictor for Neoadjuvant Chemotherapy in ER-Positive, HER2-Negative Breast Cancer

Brase JC, Gehrmann MC, Petry C, Weber KE, Schmidt M, Kobl H, Brauch H, Schwab M, Muller J, Janicke F, Roda A, Kaufmann M, Filippis M, Grant M, Denkert C, Loibl S, von Minckwitz G, Kronenwett R. Svidon Diagnostics, Cologne, Germany; Bayer Technology Services GmbH, Leverkusen, Germany; University of Mainz, Germany; Dr. Margarete Fischer-Bosch Institute of Clinical Pharmacology Stuttgart and University Tubingen, Germany; University Medical Center Hamburg-Eppendorf, Germany; J.W. Goethe University, Frankfurt, Germany; Medical University of Vienna, Austria; Charite - University of Berlin, Germany; German Breast Group, Neu-Isenburg, Germany.

Prognosis/Response Predictions: Biomarkers – Methods

P1-07-01 Comparison of Four HER2 Testing Methods in the Detection of HER2-Positive Breast Cancer: Results from the FinHer Study Cohort

Huang W, Wirtz R, Weidler J, Lie Y, Sherwood T, Leinenho M, Bono P, Isola J, Kellokumpu-Lehtinen P-L, Joensuu H. Monogram Biosciences Inc., So. San Francisco, CA; STRATIFYER Molecular Pathology GmbH, Cologne, Germany; Pharma, Turku, Finland; Helsinki University Central Hospital, Helsinki, Finland; Institute of Medical Technology, University of Tampere and Tampere University Hospital, Tampere, Finland; Tampere University Hospital, Tampere, Finland.

P1-07-02 Discordance between Central and Local Laboratory HER2 Testing from a Large HER2-Negative Population in VIRGO, a Metastatic Breast Cancer Registry

Vogel CL, Bloom K, Burris H, Galraw JR, Mayer M, Pegam M, Rugo HS, Swain SM, Yandley DA, Chau M, Lalla D, Brammer MG, Kaufman PA. Sylvester Comprehensive Center at Deerfield, Miller School of Medicine, University of Miami, Miami, FL; Clariant, Inc., Aliso Viejo, CA; Sarah Cannon Research Institute, Nashville, TN. Tennessee Oncology, PLLC, Nashville, TN; University of Washington Medical Oncology, Seattle Cancer Care Alliance, Seattle, WA; Patient Advocate, New York, NY; University of California San Francisco Comprehensive Cancer Center, San Francisco, CA; Washington Cancer Institute, Washington Hospital Center, Washington, DC; Genentech, Inc., South San Francisco, CA; Norris Cotton Cancer Center, Dartmouth-Hitchcock Medical Center, Lebanon, NH.

P1-07-03 Preanalytical Variables Affect Protein Expression in Formalin Fixed Paraffin Embedded Tissue - Assessment of Intrinsic Controls To Define Tissue Quality for Immunohistochemical Analysis

Neumeister VM, Lostritto K, Siddiqui S, Anagnostou V, Vasiliakopoulou M, Zarrella EA, Molinaro AR, Hicks DG, Rimm DL. Yale University School of Medicine, New Haven, CT; Rochester University, School of Medicine, Rochester, NY.

P1-07-04 Gene Expression Module Biomarkers To Stratify Multiple Clinical and Therapeutic Endpoints for Universal Breast Cancer Companion Diagnostic


P1-07-05 HER2 Status Resolution in FISH and IHC “Double Equivocal” Breast Carcinomas by Quantitative Real-Time PCR

Porter BP, Wang Z, Downs-Kelly E, Budd GT, Lanigan C, Tubbs RR. Cleveland Clinic, Cleveland, OH; Taussig Cancer Center, Cleveland Clinic, Cleveland, OH.

P1-07-06 High Concordance of Protein (by IHC), Gene (by FISH; HER-2 Only) and Microarray Readout (by TargetPrint) of ER/PR/HER2: Results from the MINDACT Trial

Viale G, Bogaerts J, van’t Veen L, Rutgers E, Piccart M, de Snoo F, Engelen K, Russo L, Dell’Orto F, Glas A, Cardoso F, on behalf of the TRANSBIG Consortium & the MINDACT Investigators. European Institute of Oncology, Milan, Italy; European Organisation of Research and Treatment of Cancer, Brussels, Belgium; Netherlands Cancer Institute, Amsterdam, Netherlands; Institute Jules Bordet, Brussels, Belgium; AgendiaNV, Amsterdam, Netherlands, Champalimaud Cancer Center, Lisboa, Portugal.

P1-07-07 Assessing Two Methods of Meta-Analysis in Studies of Patients with Breast Cancer: Individual Patient Data-Based (IPD) Versus Literature Based Abstracted Data (AD) in 5 Meta-Analyses Including over 28,000 Patients. Are There Results Differences of Concern?

Gralla RJ, Brita E, Raftopoulos H, Spirduso I, Giannarelli D, Cognetti F. Hofstra North Shore - LI School of Medicine, Lake Success, NY; Regina Elena National Cancer Institute, Rome, Italy.
P1-07-08  PCR Genomic Grade in Breast Cancer: A New Tool for Daily Practice

P1-07-09  Estrogen Receptor (ER) mRNA and ER-Related Gene Expression in Breast Cancers That Are 1%-10% ER-Positive by Immunohistochemistry
Iwamoto T, Booser D, Valero V, Murray JL, Koenig K, Esteva FJ, Ueno NT, Zhang J, Shi W, Qi Y, Matsuoka J, Hortsobagyi GN, Haitz C, Symmans WF, Pusztai L. The University of Texas MD Anderson Cancer Center, TX; Okayama University, Okayama, Japan; Nueva Biosciences Inc, MA.

P1-07-10  Validation of a Diagnostic Molecular Signature (EHT Dx14) on Fine-Needle Aspirate Samples from Breast Tumors

P1-07-11  Consistency and Control in Clinical Assay Technology over Time: The Oncotype DX Recurrence Score and Assessment of Single Gene Expression Levels

P1-07-12  Assessment of Real World HER2 Status by Immunohistochemistry (IHC) and Fluorescence In Situ Hybridization (FISH) in Breast Cancers: Comparison with HERMark®, a Validated Quantitative Measure of HER2 Protein Expression
Huang W, Paquet A, Sivaraman S, Pesano R, Goodman L, Shenwood T, Lie Y, Hickey J, Walworth C, Haddad M, Anderson S, Bates M, Weidler J. Monogram Biosciences Inc, South San Francisco, CA; Incyte Corporation, Wilmington, DE; Quest Diagnostics Nichols Institute, San Juan Capistrano, CA; Affymetrix, Santa Clara, CA; Gilead Sciences, Inc, Foster City, CA; Cepheid, Sunnyvale, CA.

P1-07-13  Efficiency of a Laboratory Developed HER2 FISH Test on Circulating Tumor Cells

P1-07-14  Quantum Dot-Labelled Antibodies To Assess HER2 Expression in Breast Cancer
Zona S, Blackbaum E, Hjojatoleslami AS, Brown IR, Gulwic WJ. University of Kent, Canterbury, Kent, United Kingdom.

Mizuno Y, Takayanagi H, Sato K. Tokyo-West Tokushukai Hospital, Akishima-city, Tokyo, Japan.

P1-07-16  Number Needed To Count: A Novel Model for Ki67 Assessment in Breast Cancer
Bendahl P-O, Romero Q, Grabau D, Borgquist S. Institution of Clinical Sciences, Lund University, Sweden; Institution of Clinical Sciences, Lund University, Sweden.

P1-07-17  Multiplex Plasma Biomarkers Associated with Bone Metastasis from Breast Cancer
Leitzel K, All S, Bisen A, Lomakin A, Simonyan V, Lipton A. Penn State Hershey Medical Center, Hershey, PA; Lebanon VA Medical Center, Lebanon, PA; Massachusetts Institute of Technology, Cambridge, MA; BioCompanion LLC, Rockville, MD.

P1-07-18  Expanding an Online Tool for Genome-Wide Validation of Survival-Associated Biomarkers in Breast and Ovarian Cancer Using Microarray Data of 3,862 Patients
Gyorffy B, Lanczky A, Szallasi Z. Semmelweis University, Budapest, Hungary; Harvard Medical School, Boston.

P1-07-19  Analysis of HER2-Status in Breast Cancer by Mass Spectrometry in Archival, Formalin-Fixed Tissues
Sanders M, Sprang R, Harr A, Sanchez V, Manning S, Anteaga C, Liebler D. Vanderbilt University Medical Center, Nashville, TN.

P1-07-20  Consistent High False Negative Rate of HER2 qRT-PCR of Oncotype DX® in Comparison to ASCO/CAP Recommended Combined IHC/FISH Method
Dabbs DJ, Bhargava R. Magee-Women’s Hospital of UPMC, Pittsburgh, PA.

P1-07-21  Analysis of Molecular Markers by Immunohistochemistry (IHC) Method on Formalin Fixed Paraffin Embedded (FFPE) Tissues Could Predict Shorter Recurrence Free Survival (RFS) and Overall Survival (OS) among Patients Who Have Received Adjuvant Chemotherapy for Early Breast Cancer
Moe M, Gee J, Finlay P, Mansel R, Adams R. Singleton Hospital, Swansea, United Kingdom; Velindre Hospital and Cardiff University, Cardiff, United Kingdom; Cardiff University, Cardiff, United Kingdom.

P1-07-22  A Venezuelan Study of Breast Cancer Estrogen Receptor, Progesterone Receptor and HER2 Receptor Expression by the Standard Method, Immunohistochemistry (IHC), Compared to a New Method, Quantitative Reverse Transcription Polymerase Chain Reaction (RT-PCR)
Marin C-EM, Ramirez AC, Baehner FL, Yoshizawa C, Acosta MM. Fundacion BADAN, Caracas, Miranda, Venezuela; Genomic Health, Redwood City, CA.

P1-07-23  Absolute Quantification of Estrogen Receptor alpha in Breast Cancer

P1-07-24  Quantitative and Immunohistochemical Detection of Breast Cancer Cells in Blood Samples

P1-08-01  Survival in Metastatic Breast Cancer (MBC): No Evidence for Improved Survival Following Distant Recurrence after Adjuvant Chemotherapy
Tevaanwerk AJ, Gray R, Schneider BP, Smith HL, Wagner LI, Miller KD, Sparano JA. University of Wisconsin-Carbone Cancer Center, Indiana University-Simon Cancer Center, Northwestern University, Chicago, IL; Dana-Farber Cancer Institute, Research Advocacy Network; Albert Einstein University-Monffore Medical Center.

P1-08-02  Pre-Diagnosis Body Mass Index and Breast Cancer Prognosis and Survival: Report from the after Breast Cancer Pooling Project
Kwak ML, Chen WY, Weltzien E, Beasley JM, Lu W, Nechuta SJ, Querebbery CP, Prence JP, Shu XO, Caan BJ. Kaiser Permanente Division of Research; Brigham and Women’s Hospital and Harvard Medical School; Fred Hutchinson Cancer Research Center; Shanghai Institute of Preventive Medicine; Vanderbilt University; University of California, San Diego.

P1-08-03  Huge Improvement in Relapse-Free Breast Cancer Survival over the Last 25 Years
Geurts SME, van Dijk JAAM, de Veet F, Paraguay Y, Siesling S, Verbeek ALM, Tjan-Heijnen VCG. Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands; Hospital Bernhoven, Oss, Netherlands; Comprehensive Cancer Centre, Netherlands; Maastricht University Medical Centre, Maastricht, Netherlands.
**P1-08-04** Obesity, Adjuvant Therapy, and Survival Outcomes in Early-Stage Breast Cancer
Ina-Lisentorp S, Wang T, Rimawi MF, Nangia JR, Schiff A, Giordano SH, Pollak MN, Chenault CC, Osborne CK, Hilsenbeck SG. Baylor College of Medicine, Houston, TX; M.D. Anderson Cancer Center, Houston, TX; McGill University, Montreal, Canada.

**P1-08-05** Age and Survival in Women with Early Stage Breast Cancer: An Analysis Controlling for Tumor Subtype
Partidges AH, Hughes ME, Otterson R, Wong Y-N, Edge SB, Theriault RL, Blayney DW, Nalind JC, Winer EP, Weeks JC, Tamimiri RM. Dana-Farber Cancer Institute, Boston, MA; City of Hope, Duarte, CA; Fox Chase Cancer Center, Philadelphia, PA; Roswell Park Cancer Institute, Buffalo, NY; University of Texas MD Anderson Cancer Center, Houston, TX; Stanford Cancer Center, Palo Alto, CA; Brigham and Women's Hospital, Boston, MA.

**P1-08-06** Breast Cancer among Patients with Diabetes, Obesity and Abnormal Blood Lipids - A Population-Based Register Study in Sweden

**P1-08-07** Survival in US Women Following an Indication of Metastatic Breast Cancer Diagnosis and Chemotherapy Initiation: A SEER-Medicare Analysis
Rugo H, Taylor D, Sanon M, Clemmons K, Balu S, Faria C, Teitelbaum A. University of California San Francisco, OptumInsight; Eisa Inc.

**P1-08-08** Higher Prediagnostic Serum 25-Hydroxyvitamin D Is Associated with Substantially Lower Incidence of Breast Cancer: Prospective Study
Mohr SB, Garrah EM, Garland CF, Grant WB, Baggerly L. University of California San Diego, La Jolla, CA; SUNLIGHT and Nutrition Research Center, San Francisco, CA; GrassrootsHealth, San Diego, CA.

**P1-08-09** Increased Mortality in Swedish Women Diagnosed with Breast Cancer during and Shortly after Pregnancy
Lambre M, Johansson ALV, Andersson TM-L, Chatingius S, Hsieh C-C. Karolinska Institutet, Stockholm, Sweden; University of Massachusetts Medical School, Worcester; Regional Oncologic Center, Uppsala, Sweden.

**P1-08-10** Invasive Lobular Breast Cancer - No Increased Risk of Contralateral Disease
Langlands F, Horgan K, Kearns O, Burns R, Dodwell D. The General Infirmary at Leeds, Leeds, United Kingdom; The University of Birmingham, Birmingham, United Kingdom; Level 6, Bexley Wing (Institute of Oncology), Leeds, United Kingdom; St James University Hospital, Leeds, United Kingdom.

**P1-08-11** Differences in Recurrence Dynamics between Lobular and Ductal Invasive Breast Cancer
Siedling S, Kwas ABG, Grandjean I, Ho V, van der Sanger MJC, Menke-Pluymers MBE, Tian-Heijnen VCG. Comprehensive Cancer Centre the Netherlands, Utrecht, Netherlands; Catharina Hospital, Eindhoven, Netherlands; Erasmus Medical Centre, Rotterdam, Netherlands; Maastricht University Medical Centre, Maastricht, Netherlands.

**P1-08-12** Hormonal Therapy Compliance and Mortality in Metastatic Breast Cancer
Baron TI, Kennedy MJ, Sharp L, Bennett K. Trinity College, University of Dublin, Dublin, Ireland; St James' Hospital and Trinity College Dublin, Dublin, Ireland; National Cancer Registry Ireland, Cork, Ireland.

**P1-08-13** Determinants of Risk, Characteristics and Prognosis of Breast Cancer Occurring after Hodgkin Lymphoma

**P1-08-14** Arsenic Methylation Is Associated with Breast Cancer Risk in Northern Mexico
Lopez-Carrillo L, Hernandez-Ramirez U, Torres-Sanchez L, Gandolfi J, Ornelas-Aguirre JM, Cebreros-ME. Mexico National Institute of Public Health, Cuernavaca, Morelos, Mexico; University of Arizona, Tucson, AZ; Hospital de Especialidades Instituto Mexicano del Seguro Social, Cd. Obreron, Sonora, Mexico; Centro de Investigation y Estudios Avanzados (Cinvestav), Mexico DF, Mexico.

**P1-08-15** Pattern of Cardiac Monitoring and Risk of Trastuzumab Associated-Cardiac Dysfunction in a Clinical Practice: A Population Based Study
Lee-Ying R, Ubhi C, Roberts S, Lim H, Bhatt H, Gesy K, Ahmed S. University of Saskatchewan, Saskatoon, SK, Canada; University of Saskatchewan; Saskatchewan Cancer Agency.

**P1-08-16** Benign Breast Disease (BBD) and Breast Cancer in African American Women
Fehmi RA, Cote M, Ruttenber J, Alish B, Bandyopadhyay S, Alibashti B, Frost M, Hartmann L, Visscher D. Wayne State University/ Karmanos Cancer Institute/DMIC, Detroit, MI; Wayne State University/ Karmanos Cancer Institute, Detroit, MI; Mayo Clinic Cancer Center, Rochester, MN.

**P1-08-17** Pregnancy-Associated Breast Cancer Does Not Have a Worse Outcome in the 4912 Women with Breast Cancer of the AMAZONA Project
Liedeke PER, Szymonifika J, Simon SD, Banios CH, Bines J, Finkelstein D, Goss PE. Massachusetts General Hospital, Boston, MA; Universidade Federal de São Paulo (UNIFESP), São Paulo, Brazil; Pontificia Universidade Católica do RS (PUCRS), Porto Alegre, Rio Grande do Sul, Brazil; Instituto Nacional do Cancer (INCA), Rio de Janeiro, Brazil; Brazilian Breast Cancer Study Group (GBECAM), São Paulo, Brazil.

**P1-08-18** Aspirin Exposure and Nodal Status at Diagnosis in Women with Stage I-III Breast Cancer
Baron TI, Sharp L, Bennett K, Visvanathan K. Trinity College, University of Dublin, Dublin, Ireland; National Cancer Registry Ireland, Cork, Ireland; Johns Hopkins School of Public Health, Baltimore, MD.

**P1-08-19** Withdrawn

**P1-08-20** Parity Interferes with the Effect of Age at Diagnosis on the Frequency Breast Cancers Are Triple-Negative

**P1-08-21** Demographic and Clinical Characteristics of Metastatic Breast Cancer Patients and Biomarker-Based Prevalence in the UK, Germany, France, Spain and Italy (EUROBRECA) Study
Garguili A, Diefkens M, Bonthapally V, Lee WC, Ray S, Abbott Laboratories, Abbott Park, IL; IMS Consulting Group, Alexandria, VA.

**P1-08-22** Treatment Patterns and Clinical Outcomes in Elderly Patients with HER2-Positive Metastatic Breast Cancer from the registHER Observational Study
Kaufman PA, Brujulis AM, Mayer M, Rugo HS, Tripathy D, Ulicicjko Yood M, Feng S, Wang LL, Brammer MG, Yardley DA. Norris Cotton Cancer Center, Dartmouth-Hitchcock Medical Center, Lebanon, NH; University of Pittsburgh Cancer Center, Pittsburgh, PA; Patient Advocate, New York, NY; University of California San Francisco Comprehensive Cancer Center, San Francisco, CA; University of Southern California/Norris Comprehensive Cancer Center, Los Angeles, CA; EpiSource, LLC, Boston, MA; Boston University School of Medicine, Boston, MA; Genentech, Inc., South San Francisco, CA; Sarah Cannon Research Institute, Nashville, TN; Tennessee Oncology, PLLC, Nashville, TN.
P1-08-23 Characteristics of De Novo Stage IV Breast Cancer Presentation and Comparison with Stage IV Disease Relapse after Adjuvant Therapy

P1-08-24 Clinical and Pathologic Characteristics of Diabetic Breast Cancer Patients in a Tertiary Care Safety Net Hospital
Loch MM, Eapen A, Ross AA, Rosenberg C, Blanchard RA. Boston University Medical Center, Boston, MA; Boston University, Boston, MA.

P1-08-25 Breast Cancer Burden, Risks and Outcomes in Latin America

P1-08-26 Assessment of the impact of ineffective treatments on metastatic breast cancer - a US managed care perspective
Montero AJ, Eapen S, Gorin B, Waryas CM, Adler P. Sylvester Comprehensive Cancer Center, University of Miami, Miami, Florida; Analysis Group, Inc, Boston, MA; Veriindex, LLC, Ranjan, NJ.

P1-08-27 Assessment of the economic burden of metastatic breast cancer - a US managed care perspective
Montero AJ, Eapen S, Gorin B, Waryas CM, Adler P. Sylvester Comprehensive Cancer Center, University of Miami, Miami, Florida; Analysis Group, Inc, Boston, MA; Veriindex, LLC, Ranjan, NJ.

P1-08-28 Differences in long-term survival for Hispanic and non-Hispanic white women with breast cancer
Baumgartner KB, Pinkston CM, Denhoff SR, Baumgartner RN. School of Public Health & Information Sciences, University of Louisville, Louisville, KY.

Epidemiology, Risk, and Prevention: Epidemiology – Genetic and Molecular

P1-09-01 Effect of Obesity on Gene Expression in Invasive Breast Tumors
Ellsworth RE, Croft DT, Ellsworth DL, Shriver CD, Henry M. Jackson Foundation, Windber, PA; Windber Research Institute, Windber, PA; Walter Reed Army Medical Center, Washington, DC.

P1-09-02 HDL-Cholesterol and Low-Penetrance Gene CYP17 rs2486758 Influence Daily Estrogen Levels. The EBBa-I Study
Iversen A, Thune I, McTienan A, Makart MV, Wilsgaard T, Ellison PT, Jasienska G, Floro V, Poole E, Furburg A S. University of Tromsø, Tromsø, Norway; Oslo University Hospital, Oslo, Norway; Fred Hutchinson Cancer Research Center, Seattle, WA; Harvard University, Cambridge, MA; Medical College, Krakow, Poland; Channing Laboratory, Boston, MA; University Hospital of North Norway, Tromsø, Norway.

P1-09-03 Prevalence of Germline TP53 Mutations in Young Women with HER2-Positive Breast Cancer
Dick MG, Mascian S, Miron A, Miron P, Foley K, Gelman R, Dillon DA; Richardson AL, Versels SJ, Lypas G, Krop IE, Garber JE. Dana Farber Cancer Institute, Boston, MA; Brigham and Women’s Hospital, Boston, MA.

P1-09-04 A Genetic Predictor for Breast Cancer Risk in a Japanese Population
Sueta A, Ito H, Iwata H, Hosono S, Watanabe M, Iwase H, Tajima K, Tanaka H, Matsuoka K. Aichi Cancer Center Research, Nagoya, Japan; Kumamoto University Graduate School of Medical Science, Kumamoto, Japan; Aichi Cancer Center Central Hospital, Nagoya, Japan; Nagoya University Graduate School of Medicine, Nagoya, Japan.

P1-09-05 A 3'UTR Functional Variant in BRCA1: A Predictor of Poor Outcome in Breast Cancer
Donaraj JJ, Miller N, Newell J, Kerin MJ, Weedhaas JB. National University of Ireland, Galway, Ireland; Yale University, New Haven, CT.

P1-09-06 Single Nucleotide Polymorphisms in the BRMS1 and SIPA1 Metastasis Suppressor Genes as Prognostic Markers in Breast Cancer Patients
Roberts MR, Hong C-C, Edge SB, Yao S, Nesline M, Ambrosone CB. Roswell Park Cancer Institute, Buffalo, NY.

P1-09-07 Contribution of TP53 p.R337H Mutation to Breast Cancer Incidence in Brazil

P1-09-08 Association of Hypoxia-Inducible Factor-1 with Breast Cancer Risk: A Meta-Analysis of Published Studies
Yin W, Liu G, Lu J, Shen Z, Shao Z. Fudan University Shanghai Cancer, Shanghai, China.

P1-09-09 Fetal Microchimerism and In Situ Breast Cancer
Eur JG, Kadi P. University of Washington, Seattle, WA; Fred Hutchinson Cancer Research Center, Seattle, WA.

P1-10-01 Utility of Routine Cardiac Ejection Fraction (CEF) Measurement Prior to Anthracycline-Based Chemotherapy (ABC): A Study of 466 Patients with Early-Stage HER2-Negative Invasive Breast Cancer
Policenati SM, Go RS, De Maiffe BM, Gudmun JD, McHugh VL. Gundersen Lutheran Medical Foundation, La Crosse, WI; Gundersen Lutheran Medical Centre, La Crosse, WI; Gundersen Lutheran Medical System, La Crosse, WI.

P1-10-02 Burden of Brain Metastases in HER2-Positive Breast Cancer: Healthcare Use and Costs from a French Observational Retrospective Multicenter Study
Baffert S, Cottu PH, Kirova Y, Bachetot T, Le Rhen E, Mercier F, Levy C, Guitierrez M, Madrange N, Moldovan C, Guiu S, Serin D, Cotte FE, Benjamin L, Simondi C, Mailhard C, Laihure-Vigné S, Durand-Zaleski I. Institut Curie, Paris, France; Centre Léon Bérard, Lyon, France; Centre Oscar Lambret, Lille, France; Stat Process, Fort Mort, France; Centre François Baclesse, Caen, France; Institut Curie; Saint-Cloud, France; Institut Bergoné, Bordeaux, France; Centre Henri Becquerel, Rouen, France; Centre GF Leclerc, Dijon, France; Institut Sainte Catherine, Avignon, France; GlaxoSmithKline, Marly Le Roi, France; Ceri Medical, Garches, France; CHU Henri Mondor, Créteil, France.

P1-10-03 Colony Stimulating Factor Use with Taxane-Based Therapy for Metastatic Breast Cancer: Claims Analysis of Prophylaxis, Treatment, and Costs
Force RW, Pugimire BA, Culbertson VL, ImproveRX, LLC, Pocatello, ID; Idaho State University, Pocatello, ID.

P1-10-04 Cost-Utility of the 21-Gene Breast Cancer Assay (Oncotype DX®) in the Irish Healthcare Setting
Lacey L, Chien R, Homberger J. Lacey Solutions Ltd, South Strand, Skerries, County Dublin, Ireland; Cedar Associates LLC, Menlo Park, CA; Columbia University, New York, NY; Stanford University, Stanford, CA.

P1-10-05 Is the 21-Gene Breast Cancer Test (Oncotype DX®) Cost-Effective?
Pronzato P, Plun-Favre J. Istituto Naz.le Ricerca Cancro, Genova, Italy; Genomic Health International Srl, Geneva, Switzerland.

P1-10-06 Economic Analysis of Chemotherapy Costs for Adjuvant Therapy in Breast Cancer in France
P1-10-07  Comparing Breast Cancer Screening Guidelines: A Stage-Survival-Cost Model in a Public Hospital
Friedman DT, Baskind-Had C, Adams K, Beck e E, Habets I, D’Oni C, Gundry K, Birdsong G, Gabram-Mendola S. Emory University, Atlanta, GA.

Psychosocial, Quality of Life, and Educational Aspects: Disparities and Barriers to Care

P1-11-01  Strong Socioeconomic Disparities in Breast Cancer Quality of Care in Switzerland

P1-11-02  Racial/Ethnic Differences in Adjuvant Trastuzumab Receipt for Women with Breast Cancer within the National Comprehensive Cancer Network
Freedman RA, Hughes ME, Ottesen RA, He Y, Weeks JC, Wong Y-N, Theriault RL, Keating NL. Dana-Farber Cancer Institute, Boston, MA; City of Hope, Duarte, CA; Harvard Medical School, Boston, MA; Fox Chase Cancer Center, Philadelphia, PA; University of Texas MD Anderson Cancer Center, Houston, TX; Brigham and Women’s Hospital, Boston, MA.

P1-11-03  Breast Conserving Surgery or Mastectomy - An Urban Bias? A Rural Surgical Patient Population and Factors That Lend to Therapeutic Decisions in Breast Cancer
Wooldridge RD, Borgstrom DC. Bassett Medical Center, Cooperstown, NY.

P1-11-04  Breast Cancer Treatment Resources and Guideline-Concordant Treatment in Appalachia
Yao N, Hillemeier MM, Anderson RT, Matthews SA. Penn State, University Park, PA; Penn State, Hershey, PA.

P1-11-05  Investigation of Epidemiological Factors as Barriers to Indicated Radiation Therapy in Post-Mastectomy Breast Cancer Patients in South Carolina
Rhome RM, Wahlgquist A, Garrett-Mayer E, Harper J. Medical University of South Carolina, Charleston, SC.

P1-11-06  Barriers to Enrollment in Cancer Therapeutic Clinical Trials: A Comprehensive Cancer Center Experience
Bourdeau L, Niland J, Stiller T, Swain-Cabiales S, Somlo G. City of Hope Medical Center, Duarte, CA.

P1-11-07  Impact of Reduction in Cost-Sharing on Screening Mammography Utilization among Rural U.S. Women

P1-11-08  Breast Cancer Screening Resources and Stage at Diagnosis in Appalachia: A Geospatial Perspective
Yao N, Hillemeier MM, Anderson RT. Penn State, University Park, PA; Penn State, Hershey, PA.

P1-11-09  Effects of a Multidisciplinary Breast Cancer Clinic in an Appalachian Based Medical Center
Ingham JA, Cremeans DK, Daugherty SL, Myhand RC, Sever WE. Adena Health System, Comprehensive Breast Care Program, Chillicothe, OH.

P1-11-10  Quality of Breast Cancer Care in a Boston Area Patient Navigator Program
Raj A, Ko N, Battaglia T, Moy B. Massachusetts General Hospital, Boston, MA; Boston Medical Center, Boston, MA.

P1-11-11  Wait Times for Breast Cancer Care in Manitoba 2009-2010. Time To Face the Challenge
Carpenter-Kellett T, Nashed M. CancerCare Manitoba, Winnipeg, MB, Canada.

P1-11-12  Patterns of Care of Newly Diagnosed Patients with Breast Cancer in Mexico
Chavarrí-Guerra Y, Ljedak PER, Syecko H, Hammond EE, Higgins MJ, Finkelstein D, Goss PE. Massachusetts General Hospital, Boston, MA.

P1-11-13  Improvement in the Quality of Care for Patients with Locally Advanced Breast Cancer through Implementation of an Integrated Electronic Care Pathway
Hoogleveen S, Han D, George RL, Sweet-Goldstein M, Dinnwell RE, Muradali D, Brezden CB, Haq R, Simmons CE. St. Michael’s Hospital; Princess Margaret Hospital.

P1-11-14  Gender and Sexual Orientation of Clients Who Were Linked to Breast Cancer Screening Services through Outreach and Education Supported by the Avon Breast Health Outreach Program

Treatment – Therapeutic Strategies: HER2-Targeted Therapy

P1-12-01  Pregnancy during and Following Adjuvant Trastuzumab in Patients with HER2-Positive Breast Cancer: An Analysis from the HERA Trial (BIG 01-01)
Azim Jr HA, Metzger-Filho O, de Azambuja E, Loibl S, Focant F, Gresko E, Procter M, Piccart-Gebhart M. Institut Jules Bordet, Brussels, Belgium; German Breast Group, Frankfurt, Germany; F. Hoffmann-La Roche, Basel, Switzerland; Frontier Science, Kincraig, United Kingdom.

P1-12-02  Patient-Reported Outcomes (PROs) from a Randomized Phase II Study (TDM4450g/BO21976) of Trastuzumab Emtansine (T-DM1) vs Trastuzumab Plus Docetaxel (HT) in Previously Untreated HER2-Positive Metastatic Breast Cancer (MBC)
Bianchi GV, Kocis J, Diix L, Tongoe Y, Lalla D, Tong YB, Guardino AE, Hurvitz SA. Fondazione IRCCS Istituto Nazionale Tumori, Milan, Italy; Semmelweis University Budapest, Budapest; Sint-Augustinus Antwerp, Genentech, UCL/A/Translational Oncology Research International.

P1-12-03  Combined Targeting of the PI3K Pathway and HER2 Overcomes Acquired and De Novo Trastuzumab Resistance
O’Brien NA, McDonald K, Von Eeuw E, Conklin D, Kalous O, Di Tomaso E, Finn RS, Slamon DJ. University of California at Los Angeles, Los Angeles, CA; Novartis-Oncology, Cambridge, MA.

P1-12-04  EBPSO - A Novel Biomarker for Resistance to Endocrine and HER2-Targeted Therapies in Breast Cancer
Gu G, Covington RR, Fernandez NM, Ando’ S, Fuqua SAW. Baylor College of Medicine, Houston, TX; University of Calabria, Arcavacata di Rende, Cosenza, Italy.

P1-12-05  Complete Pathological Response of Ductal Carcinoma In Situ after Treatment with Neoadjuvant Herceptin Chalmers CR, Mallon EA, Touqan N, Horgan KJ, MacPherson I, Doughty JC. The Western Infirmary, Glasgow, Scotland, United Kingdom; Leeds General Infirmary, Leeds, Yorkshire, United Kingdom.

P1-12-06  The Role of MAPK and PI3K/AKT/mTOR Signaling in Innate Lapatinib Resistance
McDermott M, O’Brien N, McDonald K, Crown J, O’Donovan N, Slamon D. Dublin City University, Glasnevin, Dublin 9, Ireland; University of California Los Angeles, Los Angeles, CA; St. Vincents University Hospital, Elm Park, Dublin 4, Ireland.

P1-12-07  Pharmacokinetics (PK) of Trastuzumab Emtansine and Paclitaxel or Docetaxel in Patients with HER2-Positive MBC Previously Treated with a Trastuzumab-Containing Regimen
Lu D, Modi S, Elias AD, Agarwal P, Yi H, Guardino AE, Athaous BL, Ginsh S. Genentech, South San Francisco, CA; Memorial Sloan-Kettering Cancer Center, New York, NY; University of Colorado, Aurora, CO.
P1-12-08 Induction of Adhesion Molecules on Target Cells Maximizes Antibody-Dependent Cell-Mediated Cytotoxicity of Anti-Her2 Therapeutic Antibody with High Fucosyl Saccharide Jia X, Wong P-Y, Gazzano-Santojo H. Genentech Inc., South San Francisco, CA.

P1-12-09 Safety and Efficacy of Naretinib in Combination with Cephalotaxine in Patients with IB2-Positive Breast Cancer Saura C, Garcia-Saizco JA, Xu B, Harb W, Morosoe P, Piuard T, Kiger C, Germa C, Wang K, Kim S-B. Breast Cancer Unit, Vall d’Hebron University Hospital, Barcelona, Spain; Hospital Clinico San Carlos, Madrid, Spain; Cancer Hospital, Chinese Academy of Medical Sciences, Beijing, China; Horizon Oncology Center, Lafayette, IN; Florida Hospital Cancer Institute, Orlando, FL, Washington University School of Medicine, St Louis, MO, Pfizer Global Research and Development, Paris, France, Pfizer Inc, Pearl River, NY, Asan Medical Center, Seoul, Korea.

P1-12-10 Phase II Study Evaluating Laptuzumab (L) in Combination with Albumin Bound Paclitaxel (ab-Pac) in Women Who Have Received 0-1 Chemotherapy Regimen for HER2 Overexpressing (HER2+) Metastatic Breast Cancer (MBC) Yardley D, Hart T, Bosserman L, Saleh MN, Waterhouse DM, Richards P, Hagan MK, Del Silvio ML, Mahoney JM, Nagy M, Sarah Cannon Research Institute; Tennessee Oncology, PLLC; Florida Cancer Specialists; Willshire Oncology Medical Group; Georgia Cancer Specialists, Oncology & Hematology Care, Inc.; Virginia Cancer Care, Oncology & Hematology Associates of SW, GlaxoSmithKline, Collegeville, PA.

P1-12-11 Neoadjuvant Chemotherapy-Trastuzumab Versus Neoadjuvant Chemotherapy Followed by Post-Operative Trastuzumab: A Multicentre Study Palmieri C, Yan K, Owadally W, Shah D, Gogis Q, North B, Riddle P, Ahmad R, Lewanski C, Coombes RC, Cleator S, Howell S, Beresford M. Imperial College Healthcare NHS Trust, London, United Kingdom; Bristol Haematology and Oncology Centre, Bristol, United Kingdom; Third Faculty of Medicine, Charles University, Prague, Czech Republic; Imperial College London, London, United Kingdom; The Christie NHS Foundation Trust, Manchester, United Kingdom.

P1-12-12 Evaluation of Ile655Val HER2 Polymorphism Associated with Cardiac Toxicity Following the Administration of Trastuzumab in Women with Non-Metastatic Breast Cancer Dinoia C, Lermieux J, Côté M-A, Provencher L, Nadeau-Larochelle C, Jacob S, Demers É, Tremblay-Lemay R, Saint-Pierre C, Beauchemin M, Barabé F, Laflamme C. Centre de Recherche FRSQ du CHA de Laval, Laval, Quebec; Quebec City, QC; Canada; Hospital du Saint-Sacrement, Quebec City, QC, Canada; Université Laval, Quebec City, QC, Canada; Hospital of the Saint-Sacrement, Quebec City, QC, Canada; Centre Hospitalier de l’Université Laval, Quebec City, QC, Canada; Centre Hospitalier Universitaire de Québec, Quebec City, QC, Canada.

P1-12-13 Comparative Pharmacokinetics (PK) of Trastuzumab Emtansine (T-DM1) in Patients Who Have or Who Have Not Received Prior Treatment for Human Epidermal Growth Factor 2 (HER2)-Positive Metastatic Breast Cancer (MBC) Wang B, Conte P, Casanova LA, Vinholes JDF, Saad OM, Yi J-H, Gupta M, Song C, Olsen SR, Perez EA, Grish S. Genentech, South San Francisco, CA; University of Modena and Reggio Emilia, Modena, Italy; Instituto Oncologico Mafra, Lima, Peru; Clinica Porto Alegre, Brazil; Mayo Clinic, Jacksonville, FL.

P1-12-14 Genetic Ablation or Pharmacological Inhibition of Autophagy Suppresses Intrinsic Resistance of Breast Cancer to HER2-Targeted Therapies Cuff S, Oliveras-Ferraros C, Vazquez-Martin A, Sauni-Nadal T, Del Barco S, Martín-Castillo B, Lopez-Bonet E, Menendez JA. Catalan Institute of Oncology, Girona, Catalonia, Spain; Girona Biomedical Research Institute (IDIBG), Girona, Catalonia, Spain; University Hospital of Girona Dr. Josep Trueta, Girona, Catalonia, Spain.


P1-12-16 HER-2 Testing and Treatment - Is Age a Factor? Singh JK, Ewing K, Howell S, Howe M, Cramer A, Bunded NJ. School of Cancer and Enabling Sciences, University of Manchester; Paterson Institute for Cancer Research, Manchester, United Kingdom; University Hospital of South Manchester, Wythenshawe Hospital, Manchester, United Kingdom; The Christie NHS Foundation Trust, Manchester, United Kingdom.

P1-12-17 Fluorescent In Situ Hybridization Evaluation of HER2 Status in Tumors with Chromosome 17 Polysomy Pietri E, Medri L, Farolfi A, Sarti S, Maltoni R, Cecconetto L, Ibrahim T, Paioli A, Serra L, Amadori D, Rocca A. Istituto Scientifico Romagnolesi for Clinical Research (I.R.S.T), Meldola, (FC), Italy; Morgagni-Pierantoni Hospital, Forli, (FC), Italy.

P1-12-18 Change in HER2 Status in HER2 Positive Operable Breast Cancer Patients Treated with Neoadjuvant Chemotherapy with or without Anti-HER2 Therapy: Analysis of Two Consecutive Cohorts Barbieri E, Picentini F, Dieci MV, Ferrara G, Bettelli S, Conte P, Guarnieri M. Modena University Hospital, Modena, Italy.

P1-12-19 Phase I Study of Single Agent Trastuzumab Emtansine in Japanese Patients with Human Epidermal Growth Factor Receptor2 (HER2)-Positive Metastatic Breast Cancer (J022591) Aogi K, Ando M, Iwata H, Hara F, Matsubara M, Fujiwara Y. National Hospital Organization Shinokaku Cancer Center, Matsuyama, Ehime, Japan; National Cancer Center Central Hospital, Chuo-ku, Tokyo, Japan; Chuo Hospital Aichi Cancer Center, Nagoya, Aichi, Japan; Chugai Pharmaceutical Co. Ltd, Chuo-ku, Tokyo, Japan.

P1-12-20 The Safety and Tolerability of Vorniostat in Combination with Lapatinib in Advanced Solid Tumors Churmsri S, Tait NS, Mediros BM, Bauer KS, Betts K-M, Lewis JC, Bao T, Feigenberg SJ, Kesmodel SB, Steams V, Edelman ML, Sausville EA, Tkaczuk KHR. University of Maryland Greenebaum Cancer Center, Baltimore, MD; The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, MD; University of Maryland, Baltimore, MD.

P1-12-21 Adjuvant Trastuzumab Treatment Without Adjuvant Chemotherapy in Early Breast Cancer Dall P, Koch T, Lenzen G, Kuhn T, Hielscher C, Reichert D, Maasberg M, Ehscheidt P, Eustermann H, Fischer G. Städt. Klinikum, Lüneburg, Germany; Osnabrück, Germany; Brustzentrum, Stuttgart, Germany; Praxis, Stralsund, Germany; Praxis, Westerstedte, Germany; Praxis, Mayen, Germany; Praxis, Neuwied, Germany; WISP, Langenfeld, Germany; Landkreis Mittweida Khs., Mittweida, Germany.

P1-12-22 Impact on Survival of the Level of HER2/neu Gene Amplification in Patients with HER2-Positive (HER2+)
Advanced Breast Cancer (AdvBrCa) Treated with Trastuzumab (H) Gullo G, Betto D, Zuradelli M, Masci G, Giordano L, Bareggi C, Salvini P, Runza L, Santoro A. St. Vincent’s University Hospital, Dublin, Ireland; Istituto ClinicoHumanitas, Rozzano, Milano, Italy; Humanitas Cancer Centre, Rozzano, Milano, Italy; Fondazione IRCCS Ca’ Granda Ospedale Maggiore Policlinico, Milano, Italy; Cliniche Humanitas Gavazzeni, Bergamo, Italy.
P1-12-23  HER4 Coexpression Is Associated with Improved Recurrence Free Survival in HER2-Positive, Herceptin Treated Patients
Brockhoff G, Macleod A, Piendl G, Seegers S, Sassen A, Diermeier-Daucher S, Buchholz S, Ottmann O. University Medical Center
Regensburg, Regensburg, Bavaria, Germany.

P1-12-24  Adherence and Persistence with Lapatinib in Women with Metastatic Breast Cancer Who Were Previously Treated with Trastuzumab
Delea TE, Kartashov A, Sharma P. Policy Analysis Inc. (PAI), Brooklyn, MA; GlaxoSmithKline, Philadelphia, PA.

P1-12-25  Evaluation of PTEN, EGFR and Ki-67 Expression as Predictors of Response to a Trastuzumab-Containing Neoadjuvant Chemotherapy Regimen in a HER-2 Over-Expressing Locally Advanced Breast Cancer (LABC) Trial
BC, Canada; British Columbia Cancer Research Centre, Vancouver,
BC, Canada; British Columbia Cancer Agency, Kelowna, BC, Canada;
British Columbia Cancer Agency, Victoria, BC, Canada; British Columbia Cancer Agency, Surrey, BC, Canada.

P1-12-26  Global Patterns of Care for HER2/neu Overexpressing Breast Cancer

Treatment – Therapeutic Strategies: Immunotherapy

P1-13-01  An Update of a Phase II Trial of the HER2 Peptide AE37 Vaccine in Breast Cancer Patients To Prevent Recurrence
Peoples GE, Mittendorf EA. Brooke Army Medical Center, Ft. Sam Houston, TX; Saint Savas Cancer Hospital, Athens, Greece;
Naval Medical Center San Diego, San Diego, CA; Uniformed Services University of the Health Sciences, USMCI, Bethesda, MD, UT M.D. Anderson Cancer Center, Houston, TX.

P1-13-02  Long-Term Clinical Benefit of Adjuvant Breast Cancer Vaccine: 5 Year Efficacy of E75 with Multiple Booster Inoculations
Vreeland TJ, Clifton GT, Sears AK, Hale DF, Patil R, Olive KS, Holmes JP, Mittendorf EA, Ponniah S, Peoples GE. Brooke Army Medical Center, Ft. Sam Houston, TX; Windber Medical Center, Windber, PA; Naval Medical Center San Diego, San Diego, CA; UT M.D. Anderson Cancer Center, Houston, TX; Uniformed Services University of the Health Sciences, USMCI, Bethesda, MD.

P1-13-03  Zoledronic Acid Induces an Immune Response in Breast Cancer Patients through Stimulation of Central Memory and Effector Memory gamma/delta T-Cells

P1-13-04  Phase II Study of Topical Imiquimod and Abraxane for Treatment of Breast Cancer Cutaneous Metastases

Treatment – Therapeutic Strategies: Antiangiogenic Therapy

P1-14-01  Randomized Phase II Trial of Weekly vs. q 2-Weekly vs. q 3-Weekly Nanoparticle Albumin-Bound Paclitaxel with Bevacizumab as First-Line Therapy for Metastatic Breast Cancer
Seidman AD, Conlin AK, Bach A, Forero-Torres A, Wright G, Hackney MH, Clasonion A, Schofield D, Igleias J, Hudis CA. Memorial Sloan-Kettering Cancer Center, New York, NY; Providence Cancer Center, Portland, OR; University of Alabama - Birmingham, Birmingham, AL; Florida Cancer Institute, Hudson, FL; Virginia Commonwealth University, Richmond, VA; Celgene Corporation, Summit, NJ.

P1-14-02  Correlation of Circulating Tumor Cells (CTC) and Circulating Endothelial Cells (CEC) with Pathological Complete Response (pCR) during Neoadjuvant Chemotherapy (CT) Combined with Bevacizumab in HER2 Negative Inflammatory Breast Cancer (IBC): Ancillary Study of Phase II Trial BEVERLY 1
Pierga J-Y, Bidard F-C, Andre F, Petit T, Dalenc F, Delazer T, Romieu G, Bonnettere J, Ferrero J-M, Kerbrat P, Lemonnier J, Viens P. Institut Curie, Paris, France; Institut Gustave Roussy, Villejuif, France; Centre Paul Strauss, Strasbourg, France; Centre Claudius Regaud, Toulouse, France; Centre François Baclesse, Caen, France; Centre Val d’Aurelle, Montpellier, France; Centre Oscar Lambret, Lille, France; Centre Antoine Lacassagne, Nice, France; Centre Eugène Marquis, Rennes, France; UniFrance, Paris, France; Centre Paoli Calmettes, Marseille, France.

P1-14-03  AVALUZ Study: First Line with Bevacizumab in Combination with Paclitaxel (P) and Gemcitabine (G) in Patients with HER-2 Negative Recurrent or Metastatic BC: PFS Analysis
Salvador J, Ciruelos E, Codeds de Villena M, Jaen A, Gil M, Galan A, Murias J, Kara C, de la Haba J, Baena JM, Villanueva MJ, Bayo J, Blancas I, Gonzalez E, Perez D, Mel JR, Manso L. Hospital U. de Valme, Seville, Spain; Hospital 12 de Octubre, Madrid, Spain; Hospital Virgen Macarena, Seville, Spain; Hospital de Jaen, Jaen, Spain; ICO, Bellvitge, Spain; Hospital de Sagunto, Sagunto, Spain; Hospital Insular de Gran Canaria, Gran Canaria, Spain; Fundacion Hospital de Alcorcon, Alcorcon, Spain; Hospital Reina Sofia, Cordoba, Spain; Hospital Puerta del Mar, Cadiz, Spain; Hospital Meixoeiro, Vigo, Spain; Hospital Juan Ramon Jimenez, Huelva, Spain; Hospital San Cecilio, Granada, Spain; Hospital Virgen de las Nieves, Granada, Spain; Hospital Costa del Sol, Marbella, Spain; Hospital Lucas-Augusti, Lugo, Spain.

P1-14-04  Prolonged (≥1 Year) Exposure to First-Line Bevacizumab Combined with Paclitaxel in Patients with HER2-Negative Metastatic Breast Cancer Treated in a Routine Oncology Practice Study
Kuemmell S, Schneeweiss A, Foerster FG, Geberth M, Tesch H, Klare P, Schumacher C, Hollburg W, Soelings U, Schmidt M, Klassen Essen-Mitte, Essen, Germany; National Center for Tumor Diseases, Heidelberg, Germany; University of Applied Sciences Zweickau, Zweickau, Germany; SPGO-Mannheim, Mannheim, Germany; Oncological Practice Bethanien, Frankfurt, Germany; Oncology Practice Krebsklinikuale für Frauen, Berlin, Germany; St Elisabeth-Hospital, KölN, Germany; HOPA (Hämatologisch-Onkologische Praxis Altona) im Struenseehaus, Hamburg, Germany; Practice, Kassel, Germany; University Hospital Mainz, Mainz, Germany.

P1-14-05  Surgical Complications from the geparQuinto trial of patients with HER2-positive metastatic breast cancer
Steinmann et al. Universitätsklinikum, Frankfurt; German Breast Group, Neu-Isenburg; St. Barbara-Klinik, Heessen; St. Elisabeth-Hospital, Gelsenkirchen; St. Barbara-Klinik, Essen; Universitätsklinikum, Freiburg; Henrietten Stiftung, Rheinfelden; Universitätsklinikum, Greifswald; Helios Klinikum Berlin Buch, Berlin; Universitätsklinikum, Kiel; Praxisklinik, Berlin; Luisenkrankenhaus, Regensburg, Regensburg, Bavaria, Germany.

December 6-10, 2011  17s  Cancer Res; 71(24 Suppl.) December 15, 2011
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P1-15-01 Oral Fluoropyrimidine (UFT and S-1) May Augment the Efficacy of Aromatase Inhibitor Via the Down-Regulation of Estrogen Receptor in Estrogen-Responsive Breast Cancer Xenografts

P1-15-02 Nanoparticles Overcome the Decreased Responsiveness of Breast Cancer Cells to a Chemotherapeutic Drug in the Presence of Adipocytes
DeAngel RE, Sandoval MA, Lansakara-P DSS, Dunlap SM, Hursting SD, Cui Z. The University of Texas at Austin, Austin, TX; UT-MD Anderson Cancer Center, Smithville, TX.

P1-15-03 Multistage Vectored Nanotherapeutics for Breast Cancer Metastasis
Shen H. The Methodist Hospital Research Institute, Houston, TX.

P1-16-01 A Randomized, Double-Blinded, Controlled Study of Exemestane vs. Anastrozole for the First-Line Treatment of Postmenopausal Japanese Women with Hormone Receptor Positive Advanced Breast Cancer
Masuda N, Iwata H, Ohno S, Raj Y, Sato Y, Ohsumi S, Hashigaki S, Nishizawa Y, Saeki T, Noguchi S. NHK Osaka National Hospital, Osaka, Japan; Aichi Cancer Center Hospital, Aichi, Japan; Kyushu Cancer Center, Fukuoka, Japan; Saga Hospital, Kagoshima, Japan; Nagoya Medical Center, Aichi, Japan; Shikoku Cancer Center, Ehime, Japan; Pfizer Japan Inc, Japan; Saitama Medical University International Medical Center, Saitama, Japan; Osaka University, Osaka, Japan.

P1-17-01 Figitumumab Plus Exemestane Versus Exemestane as First-Line Treatment of Postmenopausal Hormone Receptor-Positive Advanced Breast Cancer: A Randomized, Open-Label Phase II Trial
Ryan PD, Nevon P, Blackwell KL, Dirix LY, Barrios CH, Miller, Jr WH, Fein LE, Fenton D, Benner RJ, Meech SJ, Paccagnella L, Sleight B, Yee D, Goss PE. Fox Chase Cancer Center, Philadelphia, PA; University Ziekenhuis Leuven, Leuven, Belgium; Duke University Medical Centre, Durham, NC; Sint-Augustinus, Onkologisch Centrum, Antwerp, Belgium; PUCRS School of Medicine, Porto Alegre, Brazil; Lady Davis Institute for Medical Research, Hebrew General Hospital, Segal Cancer Center, McGill University, Montreal, Canada; Centro Oncologico Rosano, Santa Fe, Argentina; Cross Cancer Institute, Edmonton, Canada; Pfizer Inc., Gorton, NY; University of Minnesota, Minneapolis, MN; Massachusetts General Hospital Cancer Center, Boston, MA.

P1-17-02 A Phase 1/2 Study of SAR245408 (S08) in Combination with Trastuzumab (T) or Paclitaxel (P) and T in Patients with HER2+ Metastatic Breast Cancer (MBC) Who Progressed on a Previous T- Based Regimen
Tolaney S, Burris H, Gartner E, Mayer I, Saura C, Maurel M, DeCillis A, Ruiz-Soto R, Lager J, Winger E, Krop I. Dana Farber Cancer Institute, Boston, MA; Sarah Canon Research Center, Nashville, TN; Wayne State University/Karmanos Cancer Institute Hematology/Oncology, Detroit, MI; Vanderbilt Ingram Cancer Center, Nashville, TN; Vill d'Hebron University Hospital, Barcelona, Spain; Columbia University Medical Center, New York, NY; Exelixis, San Francisco, CA; Sanofi, Cambridge, MA.

P1-17-04 Phase I Study of PARP Inhibitor ABT-888 (Veligparib) in Combination with Cisplatin and Vinorelbine for Patients with Advanced Triple Negative Breast Cancer and/or BRCA-Mutation Associated Breast Cancer
Rodler ET, Specht JM, Gadi KV, Kurland BF, Griffin MJ, Hammond JI, Gralow JR. University of Washington/Seattle Cancer Care Alliance, Seattle, WA; Fred Hutchinson Cancer Research Center, Seattle, WA.

P1-17-05 Preliminary Results of a Randomized Phase 2 Study of PD 0332991, a Cyclin-Dependent Kinase (CDK) 4/6 Inhibitor, in Combination with Letrozole for First-Line Treatment of Patients (pts) with Post-Menopausal, ER+, HER2-Negative (HER2-) Advanced Breast Cancer
Finn RS, Crown JP, Boer K, Lang I, Parikh RJ, Patel R, Schmidt M, Hagenestad C, Lim H, Pinter T, Amadori D, Chan D, Dichmann RA, Walshe J, Breazna A, Kim ST, Randolph S, Siamon DJ. University of California at Los Angeles, Los Angeles, CA; Irish Cooperative Oncology Research Group, Dublin, Ireland; Szent Morgit Korhaz, Budapest, Hungary; National Institute of Oncology, Budapest, Hungary; Comprehensive Cancer Centers of Nevada, Henderson, NV; Comprehensive Blood and Cancer Center, Bakersfield, CA; University Hospital Mainz, Mainz, Germany; Suburban Hematology-Oncology Associates, Lawrenceville, GA; British Columbia Cancer Agency, Vancouver, BC, Canada; Petz Aladar Megyei Okato Korhaz, Gyor, Hungary; Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori, Meldola, Italy; Cancer Care Associates Medical Group, Redondo Beach, CA; Central Coast Medical Oncology Corporation, Santa Maria, CA; Pfizer Oncology, La Jolla, CA; Pfizer Oncology, New York, NY.

P1-17-06 A Phase II Trial of the CDK 4/6 Inhibitor PD0332991 in Women with Advanced Breast Cancer

P1-17-07 Phase II Trial of RAD001 Plus Carboplatin in Patients with Triple-Negative Metastatic Breast Cancer

P1-17-08 A Phase II Trial of Ganetespib: Efficacy and Safety in Patients (pts) with Metastatic Breast Cancer (MBC)

P1-17-09 A Phase 1/2 Dose-Escalation Study of SAR245408 (S08) or SAR245409 (S09) in Combination with Letrozole (L) in Subjects with Hormone Receptor-Positive and HER2-Negative (HR+/HER2-) Breast Cancer (BC) Refractory to a Nonsteroidal Aromatase Inhibitor (AI)
Baselga J, Tolaney S, Hart L, Gomez P, Gartner E, DeCillis A, Ruiz-Soto R, Lager J, Burns H. Massachusetts General Hospital, Boston, MA; Dana Farber Cancer Institute, Boston, MA; Florida Cancer Specialists Drug Development Unit, Ft Myers, FL; HU Vall d'Hebron Oncology Service, Barcelona, Spain; Wayne State University/Karmanos Cancer Institute Hematology/Oncology, Detroit, MI; Exelixis, San Francisco, CA; Sanofi, Cambridge, MA; Sarah Cannon Research Center, Nashville, TN.
P1-17-10  Cabozantinib (XL184) in Patients with Metastatic Breast Cancer: Results from a Phase 2 Randomized Discontinuation Trial
Tolanev SM, Nechushhtan H, Berger R, Kurzrock R, Ron IG, Schöffski P, Awada A, Yasenchak CA, Burnis HA, Ramies DA, Shen X, Winer EP. Dana Farber Cancer Institute, Boston, MA; Hadassah Ein-Kerem Medical Centre, Jerusalem, Israel; The Chaim Sheba Medical Center, Tel Hashomer, Israel; MD Anderson Cancer Center, Houston, TX; Tel-Aviv Sourasky Medical Center, Tel-Aviv, Israel; Universitaire Ziekenhuis Gasthuisberg, Leuven, Flemish Brabant, Belgium; Institut Jules Bordet, Brussels, Belgium; Northwest Cancer Specialists, P.C., Tualatin, OR; Sarah Cannon Research Institute, Nashville, TN; Excelixis Inc., South San Francisco, CA.

Treatments – Advanced Disease: Advanced Therapy – Other

P1-18-01  Z-ACT1: Zometa Combined with Standard Therapy in Patients with Metastatic Breast Cancer Further Decreases the Proportion of Patients with CTC Counts of 5 or above
Brufsky A, Beck J, Dakil S, Hallmeyer S, Tezcan H, Yardley D, Tran D, Warsi G, Culver K. McGee Women’s Hospital of University of Pittsburgh Medical Center, Pittsburgh, PA; Highlands Oncology Group, Fayetteville, AR; Cancer Center of Kansas, Wichita, KS; Oncology Specialist, SC, Park Ridge, IL; Kootenai Cancer Center, Post Falls, ID; Sarah Cannon Research Institute, Nashville, TN; Tennessee Oncology, PLLC, Nashville, TN; Novartis Pharmaceuticals Corporation, East Hanover, NJ.

P1-18-02  Descriptive Analysis of the Management of Breast Cancer Patients with a Solitary Lesion Diagnosed with 18-Fluorodeoxyglucose Positron Emission Tomography/Computed Tomography Scan

5:00 pm–7:00 pm
POSTER DISCUSSION I: ENDOCRINE RESISTANCE
Ballroom A

Viewing 5:00 pm
Discussion 5:30 pm

Discussant: Suzanne A.W. Fuqua, PhD

 Baylor College of Medicine

Houston, TX

PD01-02  Randomized Phase II Study of Dasatinib vs Placebo in Addition to Exemestane in Advanced ER/PR-Positive Breast Cancer (BMS CA180-261 Study)
Lombart A, Ravaudo A, Strauss L, Sy O, Abrahao F, Geese WJ, Lortholary A, Rea D, Ro J-S, Sohn J, Kim S-B, Curigliano G. Hospital Universitari Arnau De Vilanova, Lleida, Spain; Ospedale degli Infermi di Rimini, Rimini, Italy; Bristol-Myers Squibb, Wallingford, CT; Centre Catherine De Sienne, Nantes, France; City Hospital, Birmingham, United Kingdom; National Cancer Center, Gyeonggi-Do, Korea; Yonsei Cancer Center, Seoul, Korea; Asan Medical Center, Seoul, Korea; Instituto Europeo di Oncologia, Milano, Italy.

PD01-03  Src Is a Potential Therapeutic Target in Endocrine Resistant Breast Cancer Exhibiting Low Estrogen Receptor (ER)-Mediated Transactivation
Guest SK, Pancholi S, Patani N, Dowsett M, Johnston SR, Martin L-A. Institute of Cancer Research, London, United Kingdom; Royal Marsden Hospital, London, United Kingdom.

PD01-04  Entinostat, a Novel Histone Deacetylase Inhibitor, Added to Exemestane Improves PFS in Advanced Breast Cancer in a Randomized, Phase II, Double-Blind Study
Yardley DA, Ismail-Khan RR, Klein PM. Sarah Cannon Research Institute, Nashville, TN, Tennessee Oncology, PLLC, Nashville, TN; H. Lee Moffitt Cancer Center, Tampa, FL; PMK Consulting, San Francisco, CA.

PD01-05  Histone Deacetylase Inhibitor LBH589 (Panobinostat) Suppresses the Activated-NFκB Pathway in Acquired Aromatase Inhibitor Resistant Breast Cancer Cells
Kubo M, Kanaya N, Liu Z, Shen S. Beckman Research Institute of the City of Hope, Duarte, CA.

PD01-06  Endoxifen Exhibits Potent Anti-Tumor Activity and Regulates Different Genes Than Tamoxifen in an Aromatase Expressing MCF7 Model Resistant to Letrozole

PD01-07  AR Overexpression and Aromatase Inhibitor Resistance in Breast Cancer
Rechoum Y, Iacopetta D, Barone L, Ando’ S, Morales SF, Weigel NL, Fuqua SAW. Baylor College of Medicine, Houston, TX; University of Calabria, Calabria, Italy.

PD01-08  Heterogeneity of Lapatinib Responses in HCC1954 HER2-Overexpressing Breast Cancer Cells Revealed by Single-Cell Automated Microscopy
Hardeman KN, Tyson D, Quantra V. Vanderbilt University School of Medicine, Nashville, TN.

PD01-09  Identifying Novel Mechanisms of Resistance to Lapatinib in ERBB2+ Breast Cancer Cells through Whole Genome Mutational Analysis
Jegg A, Ward TM, Iorns E, Gallas M, Aparicio SA, Pegram MD. Braman Family Breast Cancer Institute, Sylvester Comprehensive Cancer Center, University of Miami Miller School of Medicine, Miami, FL; UBC BC Cancer Agency, Vancouver, Canada.

5:00 pm–7:00 pm
POSTER DISCUSSION II: LOCAL THERAPY
Ballroom B

Viewing 5:00 pm
Discussion 5:30 pm

Co-Discussant: Richard Crownover, MD, PhD

UT Health Science Center
San Antonio, TX

&

Co-Discussant: Ismail Jatoi, MD, PhD, FACS

UT Health Science Center
San Antonio, TX
PD02-01 Impact of Contralateral Prophylactic Mastectomy on Surgical Outcomes

PD02-02 A Decision Analysis of Contralateral Prophylactic Mastectomy in Women Undergoing Treatment for Sporadic Unilateral Breast Cancer
Lester-Coll NH, Lee J, Gogineni K, Hwang W-T, Schwartz JS, Prosnitz RG. University of Pennsylvania Perelman School of Medicine, Philadelphia, PA; Massachusetts General Hospital, Boston, MA; Abramson Cancer Center, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA.

PD02-03 The Effect of Breast Conservation Therapy vs Mastectomy on Symptoms, Physical Impairments, and Function
Kesarwala AH, Pfalzer LA, O'Meara WP, Stout NL. National Cancer Institute, Bethesda, MD; University of Michigan - Flint, Flint, MI; National Naval Medical Center, Bethesda, MD.

PD02-04 A Randomized, Prospective, Multicenter Study of the Impact of Intraoperative Margin Assessment with Adjunctive Use of MarginProbe vs. Standard of Care
Schnabel F, Tafa L, The MarginProbe Study Group. NYU Langone Medical Center, New York, NY; The AAMC Breast Center, Annapolis, MD; Dune Medical Devices.

PD02-05 MRI Phenotype and Tumor Subtype Affect Breast Conservation Eligibility and MRI Accuracy in the I-SPY 1 Trial
Mukhtar RA, Hylton N, Rosen M, The I-SPY 1 Trial Investigators, ACRIN 6657. Massachusetts General Hospital, Boston, MA; PUCRS School of Medicine, Porto Alegre RS, Brazil; Barwon Health, Geelong, Victoria, Australia; Aichi Cancer Center Hospital, Nagoya, Japan; Hospital Gregorio Maranon, Madrid, Spain; Amgen Inc, Thousand Oaks, CA; Amgen (Europe) GmbH, Zug, Switzerland; University of Sheffield, Sheffield, United Kingdom.

PD02-06 Outcomes after Total-Skin Sparing Mastectomy and Immediate Reconstruction in 657 Breasts

PD02-07 Models Predicting Non-Sentinel Node Involvement in Breast Cancer Also Predict for Regional Recurrence If the Axilla Is Not Treated
Pepels M, Vestjens H, de Boer M, Bult P, van Dijk J, Mencke M, van Diest P, Borm G, Tijn V. Maastricht University Medical Centre; Radboud University Nijmegen Medical Centre; Erasmus Medical Centre Rotterdam; University Medical Centre Utrecht.

PD02-08 Validation over Time of a Nomogram Predicting the Sentinel Node Positivity in Early Breast Carcinoma According to the Molecular Subtypes Classification

PD02-09 RTSG 9804: A Prospective Randomized Trial for “Good Risk” Ductal Carcinoma In Situ (DCIS), Comparing Radiation (RT) to Observation (OBS)
McCormick B, Winter K, Hudis C, Kuerer H, Rakovitch E, Smith B, Shah A, Germain L, Hartford A, Rashian A, Walker E, Yuen A, Strem E, Kerlin K, Vallow L, Small W, Pu A, Wilcox J, White J. Memorial Sloan Kettering Cancer Center, RTSG Statistical Center, University of Texas MD Anderson Cancer Center; Toronto-Sunnybrook Regional Cancer Centre; Massachusetts General Hospital; York Cancer Center, York, PA; L’Hotel-Dieu de Quebec; Dartmouth Hitchcock Medical Center; University of Southern California-Los Angeles; Henry Ford Hospital; Reading Hospital and Medical Center-PA; Southeast Cancer Control Consortium CCOP; Mayo Clinic Jacksonville; Northwestern University Feinberg School of Medicine; Radiological Associates of Sacramento; US Oncology, Greenville, SC; Medical College of Wisconsin.

5:00 pm–7:00 pm
POSTER SESSION ONGOING TRIALS 1
Exhibit Hall B

Bone

OT1-01-01 Prospective Clinical Trial Evaluating Efficacy of Zoledronic Acid (ZA) Prophylaxis for Prevention of Aromatase Inhibitor Associated Musculoskeletal Symptoms: ZAP-AIMSS Trial
Bardia A, Blackford A, Jeter S, Tarpinian K, Fetting JF, Miller R, Slater S, Henry NL, Giles J, Stearns V. Johns Hopkins Kimmel Cancer Center, Baltimore, MD; University of Michigan, Ann Arbor, MI; Columbia University, New York City, NY.

OT1-01-02 A Multicentre Study Assessing 12-Weekly Intravenous Bisphosphonate Therapy in Women with Low Risk Bone Metastases from Breast Cancer - The TRIUMPH Trial
Bouganim N, Hilton J, Vandenmeur L, Hopkins S, Spencer P, Robbins D, Amir E, Dent S, Milano C, Ooi D, Dranitsaris G, Clemons M. The Ottawa Hospital Cancer Center, Ottawa, ON, Canada; Princess Margaret Hospital, Toronto, ON, Canada; The Ottawa Hospital, Ottawa, ON, Canada.

OT1-01-03 A Phase 3 Randomized, Double-Blind, Placebo-Controlled Multicenter Study Comparing Denosumab with Placebo as Adjuvant Treatment for Women with Early-Stage Breast Cancer Who Are at High Risk of Disease Recurrence (D-CARE)
Goss PE, Barns CH, Bell R, Finkelstein D, Ivata H, Martin M, Braun A, Ke C, Mansi T, Braun S, Dansye R, Coleman RE. Massachusetts General Hospital Cancer Center, Boston, MA; PUCRS School of Medicine, Porto Alegre RS, Brazil; Barwon Health, Geelong, Victoria, Australia; Aichi Cancer Center Hospital, Nagoya, Japan; Hospital Gregorio Maranon, Madrid, Spain; Amgen Inc, Thousand Oaks, CA; Amgen (Europe) GmbH, Zug, Switzerland; University of Sheffield, Sheffield, United Kingdom.

OT1-01-04 NEO-ZOTAC: A Phase III Randomized Trial with Neoadjuvant Chemotherapy (TAC) with or without Zoledronic Acid for Patients with HER2-Negative Large Resectable or Locally Advanced Breast Cancer
van de Ven S, Norrest JK, Olesen DK, Liefers GJ, ten Tije A, Kessels L, van Laarhoven HWM, van Warmerdam LIC, Vriens B, van den Bosch J, van Meershoek-Kleijn Kranenburg E, van Leeuwen E, Kooij JR. Leiden University Medical Centre, Leiden, Netherlands; Amphia Hospital, Breda, Netherlands; Deventer Hospital, Deventer, Netherlands; University Medical Center St. Radboud Nijmegen, Nijmegen, Netherlands; Catharina Hospital, Eindhoven, Netherlands; Academic Hospital Maastricht, Maastricht, Netherlands; Albert Schweitzer Hospital, Dordrecht, Netherlands; BOOG Study Center, Amsterdam, Netherlands.

HER2

OT1-02-01 Phase II Neoadjuvant Trial of Anthracycline Based Regimens Followed by a Combination with Nanoparticle Alumunium-Bound Paclitaxel and Trastuzumab in Patients with Operable T2-3,N0-1,Her2 Positive Breast Cancer

OT1-02-02 HALT MBC: HER2 Suppression with the Addition of Lapatinib to Trastuzumab in HER2-Positive Metastatic Breast Cancer (LPT112515)
OT1-02-03  EGFR14299: Safety and Efficacy of an Aromatase Inhibitor (AI) in Combination with Lapatinib (L), Trastuzumab (T) or Both for the Treatment of Hormone Receptor-Positive (HR+), HER2+ Metastatic Breast Cancer (MBC)

OT1-02-04  Adjuvant Pertuzumab and Herceptin in [In][T]rial Therapy of Breast Cancer: APHINITY (BIG 4-11/B025126/TOC4939g)
vom Minckwitz G, Baselga J, Bradbury I, de Azambuja E, Scullion MJ, Ross G, Saini KS, Picart-gebhart M. German Breast Group, Neu-Isenburg, Germany; Massachusetts General Hospital, Boston, MA; Frontier Science and Technology Research Foundation, Scotland; Breast Center, Jules Bordet Institute, Brussels, Belgium; Roche, Welwyn Garden City, UK; Breast International Group Headquarters, Brussels, Belgium; Breast International Group, Brussels, Belgium.

OT1-02-05  A Phase III Clinical Trial Comparing Trastuzumab Given Concurrently with Radiation Therapy to Radiation Therapy (RT) Alone for Women with HER2-Positive DCIS Resected by Lumpectomy: NSABP B-43
Julian TB, Anderson SJ, Cobleigh MA, Szili-koukou KP, Arthur DW, Zheng P, Mamounas EP, Pajon ER, Behrens RI, Chu L, Leasure NC, Atkins JN, Polkoff J, Seay TE, McCaskill-Stevens W, Rabinovitch MJ, Ross G. National Surgical Breast & Bowel Project (NSABP, Pittsburgh, PA; Allegheny General Hospital, Pittsburgh, PA; University of Pittsburgh Graduate School of Public Health and NSABP Biostatistical Center, Pittsburgh, PA; Rush University Medical Center, Chicago, IL; Northwestern University Feinberg School of Medicine, Chicago, IL; Virginia Commonwealth University, Richmond, VA; Aultman Health Foundation, Canton, OH; Colorado Cancer Research Program, Denver, CO; Iowa Oncology Research Association, Des Moines, IA; Florida Cancer Specialists, Sarasota, FL; Reading Regional Cancer Center, West Reading, PA; SCCC-CCOP, Goldsboro, NC; Kaiser Permanente Southern California, San Diego, CA; Atlanta Regional Community Clinical Oncology Program, Atlanta, GA; National Cancer Institute, Rockville, MD; University of Colorado, Aurora, CO.

OT1-02-06  DETECT III - A Multicenter, Randomized, Phase III Study To Compare Standard Therapy Alone Versus Standard Therapy Plus Lapatinib in Patients with Initially HER2-Negative Metastatic Breast Cancer and HER2-Positive Circulating Tumor Cells
Melcher CA, Janni W, Oettmann U, Jäger B, Rack B, Müller V, Schneeweiss A, Pantic-K, Solomayer E-F, Fehm T. University Hospital Duesseldorf, Duesseldorf, Germany; University Hospital Munich, Munich, Germany; National Center for Tumor Diseases Heidelberg, Heidelberg, Germany; University Hospital Hamburg, Hamburg, Germany; University Hospital Hamburg, Homburg/Saar, Germany; University Hospital Tuebingen, Tuebingen, Germany; University Hospital Hamburg-Eppendorf, Hamburg-Eppendorf, Germany.

OT1-02-07  NSABP B-47: A Randomized Phase III Trial of Adjuvant Therapy Comparing Chemotherapy Alone (Six Cycles of Docetaxel Plus Cyclophosphamide or Four Cycles of Doxorubicin Plus Cyclophosphamide Followed by Weekly Paclitaxel) to Chemotherapy Plus Trastuzumab in Women with Node-Negative or High-Risk Node-Negative HER2-Low Invasive Breast Cancer
Fehrenbacher L, Jeong J-H, Rastogi P, Geyer CE, Paik S, Ganz PA, Land SR, Costantino JP, Swain SM, Mamounas EP, Wolmark N. National Surgical Adjuvant Breast and Bowel Project (NSABP) Operations and Biostatistical Centers; Kaiser Permanente, Northern California; University of Pittsburgh Graduate School of Public Health; University of Pittsburgh Cancer Institute, University of Pittsburgh School of Medicine; Allegheny General Hospital, Monroeville Comprehensive Cancer Center, Pittsburgh, PA; Washington Cancer Institute, Washington Hospital Center, Aultman Health Foundation.

OT1-02-08  The PERSEPHONE Trial: Duration of Trastuzumab with Chemotherapy in Women with HER2 Positive Early Breast Cancer. Changing the Randomisation Point To Address Potential Barriers to Recruitment

OT1-02-09  A Phase II Randomized Trial of Lapatinib with Either Vinorelbine or Capecitabine as First- and Second-Line Therapy for HER2-Overexpressing Metastatic Breast Cancer
Janni W, Pikiel J, Terakawa T, Kaszeska W, Papadimitriou CA, Schwedler K, Alvarez Enallo J, Caruso M, Herve RA, Lau MR, Williams LS, Briggs K, Sapunar FJ. Heinrich-Heine-Universität, Chalmers lobaricentrum, Stockholm, Sweden; Department of Medical Oncology, University Hospital Hamburg, Hamburg, Germany; Department of Breast Cancer, University Hospital Tuebingen, Tuebingen, Germany; Department of Medical Oncology, University Hospital Homburg, Homburg/Saar, Germany; Department of Medical Oncology, University Hospital Hamburg, Hamburg, Germany; Division of Medical Oncology, University Hospital Munich, Munich, Germany; Division of Medical Oncology, University Hospital Hamburg-Eppendorf, Hamburg, Germany; Department of Medical Oncology, University Hospital Hamburg, Hamburg, Germany; Department of Medical Oncology, University Hospital Hamburg, Hamburg, Germany; Breast Cancer, Department of Medical Oncology, University Hospital Hamburg, Hamburg, Germany; Department of Medical Oncology, University Hospital Hamburg-Eppendorf, Hamburg, Germany; Department of Medical Oncology, University Hospital Hamburg, Hamburg, Germany.

OT1-02-10  Phase I-II Study of HER2 Vaccination with Poly(I) : Poly(C) (Ampligen®) as an Adjuvant in Optimally Treated Breast Cancer Patients
Higgins DM, Childs J, Parker S, Guthrie KA, Ditsis ML, Salazar LG. University of Washington, Seattle, WA; Fred Hutchinson Cancer Research Center, Seattle, WA.

OT1-02-11  Mother: A Registry Developed for Women with Breast Cancer Who Have Received Trastuzumab within 6 Months Prior to Conception or during Pregnancy

OT1-02-12  Early Detection of Cardiotoxicity by Advanced Cardiac Imaging and a Novel Biomarker in Breast Cancer Patients Undergoing Chemotherapy

OT1-02-13  Cardiac Biomarkers on Trastuzumab (CABOT Trial): Determining the Cardiac Biomarker Profile in Breast Cancer Patients Receiving Adjuvant Trastuzumab Therapy
Kumar VNC, Kavakas P, Rask S, Mukherjee SD, Ellis P, Dhees-Third B. Sunnybrook Odette Cancer Centre, Toronto, ON, Canada; Juravinski Hospital, Hamilton, ON, Canada; Juravinski Cancer Centre, Hamilton, ON, Canada.

Prognostic and Predictive Markers

OT1-03-01  A Randomized Phase III Clinical Trial of Standard Adjuvant Endocrine Therapy +/- Chemotherapy in Patients (pts) with 1-3 Positive Nodes, Hormone Receptor (HR)-Positive and HER2-Negative Breast Cancer with Recurrence Score (RS) of 25 or Less: SWOG S1007
Gonzalez-Angulo AM, Barlow WE, Gralow JR, Menic-Bernstam F, Hayes DF, Moopour CM, Ramsey SD, Schott AF, Sparks DB, Albain KS, Hortobagyi GN. MD Anderson Cancer Center, Houston, TX; Cancer Research and Biostatistics, Seattle, WA; Seattle Cancer Care Alliance/University of Washington, Seattle, WA; University of Michigan, Ann Arbor; MD, Fred Hutchinson Cancer Research Ctr, Seattle, WA; University of Michigan and SWOG, Ann Arbor, MI; SWOG, San Antonio, TX; Loyola Univ Chicago Stritch School of Medicine, Maywood, IL.

OT1-03-02  SAFIR01: A Molecular Screening Trial for Metastatic Breast Cancer Patients
Andre F, Peletetekan C, Jimenez M, Ferrero, Delaloge S, Roman Roman S, Dessen P, Bonnefoi H, Gustave Roussy Institute; Univac'urie; Curie Institute; Bergonie Institute; Centre antoine lacassagne, Nice, France.
P2-01-23  Seprase Promotes the Growth and Impairs the Migratory Ability of Breast Cancer Cells
Jia J, Martin TA, Jiang W. Peking University, Beijing, China; Cardiff University School of Medicine, Cardiff, United Kingdom.

P2-01-24  Triple-Negative Breast Cancer Emerges from the Luminal Progenitor Compartment
Nogi H, Kario M, Kato K, Kawaase K, Toriumi Y, Takeyama H, Uchida K. The Jikei University School of Medicine, Tokyo, Japan.

P2-01-25  Truncated p110 ERBB2 (CTF611) Increases Migration and Invasion of Breast Epithelial Cells by Inhibiting STAT5b Activation

P2-01-26  TIMPv: a novel downstream transcriptional target gene that underpins CD146-suppressed breast tumor invasion
Ouhit A, Abdelmageed Z, Fernando A, Gaur RL, Raj MHG. College of Medicine & Health Sciences, Sultan Qaboos University, Muscat, Oman; Louisiana State University Health Sciences Center; Stanley S Scott Cancer Center, New Orleans, LA.

P2-01-27  Withdrawn

Tumor Cell Biology: Oncogenes/Tumor Suppressor Genes

P2-02-01  A Novel Inflammatory Breast Cancer-Specific Oncogene, Tazarotene-Induced Gene 1, Promotes Tumorigenicity and Invasiveness through the Receptor Tyrosine Kinase Axl
Wang X, Saslo H, Iwamoto T, Pusztai L, Gong Y, Woodward WA, Reuben JM, Hortobagyi GN, Ueno NT. Morgan Welch Inflammatory Breast Cancer Clinic, The University of Texas MD Anderson Cancer Center, Houston, TX; University of Texas MD Anderson Cancer Center, Houston, TX.

P2-02-02  Mutation of the APC Tumor Suppressor Stimulates Breast Cancer Cell Proliferation through Hyperactivation of FAK/Src/JNK Signaling
Prosperi JR, Yang FF, Goss KH. University of Chicago, Chicago, IL.

P2-02-03  MAP3K3, Amplified in Human Breast Cancer, Promotes Breast Tumor Progression and Defines Poor Prognosis
Dong J. Baylor College of Medicine, Houston, TX.

P2-02-04  Novel Functions of vps34 in Non-Transformed Epithelial Cells: Regulation of Cell Proliferation and Tumorigenesis
Su H, Xu T, Ganapathy S, Yuan Z-M. UTHealthSA, San Antonio, TX.

P2-02-05  Molecular Mechanism for Src Homology Phosphotyrosyl Phosphatase 2 Cell Motility and Migration
Hartman ZR, Agazie YM. West Virginia University School of Medicine, Morgantown, WV.

P2-02-06  Inhibition of CDK4 Phosphorylation of Smad3 Decreases Cyclin D Overexpressing Breast Cancer Cell Proliferation and Migration
Tarasewicz E, Hardy A, Straela H, Foucar C, Zelivianski S, Jerus J. Northwestern University Feinberg School of Medicine, Chicago, IL.

P2-02-07  Regulation of mTOR Signaling by Proto-Oncogene PELP1
Gonugunta VK, Cortez V, Rethman C, Nair BC, Sareddy GR, Vadlamudi RK. UTHSCSA, San Antonio, TX.

P2-02-08  Int6 Regulates Both Proteasomal Degradation and Translation Initiation and Is Critical for Proper Formation of Acini by Human Mammary Epithelium
Suo J, Snider SJ, Lloyd RE, Chang EC. BCM.

P2-02-09  TP53 Mutation Patterns in Breast Cancer Subgroups

P2-02-10  Differential Targeting of the RB-Pathway To Expand the Therapeutic Index in the Treatment of Triple Negative Breast Cancer

Tumor Cell Biology: Growth Factors

P2-03-01  Genetic Reduction of Circulating Insulin-Like Growth Factor (IGF)-I Differentially Impacts the Effects of Diet-Induced Obesity and Calorie Restriction on Mammary Tumor Progression
Ford NA, Nunez NP, Perkins SN, Hursting SD. University of Texas at Austin, Austin, TX; National Cancer Institution, Rockville, MD.

P2-03-02  An IGF1R Antibody Does Not Inhibit Growth of Tamoxifen Resistant MCF-7 Cells
Fagan DH, Yee D. University of Minnesota, Minneapolis, MN.

P2-03-03  An Insulin-Like Growth Factor I (IGF-I)-Induced Gene, Solute Carrier Family 7 Member 11 (SLC7A11)/xCT, Mediates IGF-I-Induced Biological Behaviors in Breast Cancer Cells
Yang Y, Becker MA, Yee D. University of Minnesota, Minneapolis, MN.

P2-03-04  Novel Pathways Underlying the Initiation and Transition of DCIS to IDC of HER2-Overexpressing Breast Cancer Model
Pradeppe CR, Koester W, Laurola M, Nair H, Rao R, Mills GB, Yarden Y. MD Anderson Cancer Centre, Houston, TX; Weizmann Institute of Science, Rehovot, Israel; Southwest National Primate Research Center, San Antonio, TX; University of Texas Health Science Center San Antonio, San Antonio, TX.

P2-03-05  Attenuation of TGF-beta Signaling Suppresses Premature Senescence in a p21-Dependent Manner and Promotes Oncogenic ras-Mediated Metastatic Transformation in hTERT-Immortalized Basal-Like Human Mammary Epithelial Cells
Lin S, Yang J, Elkahoul AG, Bandopadhayay A, Wang L, Cornell JE, Yeh I-T, Agyn JK, Sun L-J. University of Texas Health Science Center, San Antonio, TX; NHGRI-NIH, Bethesda, MD.

P2-03-06  Endothelin-1/Endothelin A Receptor Signalling in Breast Cancer
Tamkis D, Leece C, Gallo K, Madhukar BV, Dmitrov N. Michigan State University (MSU).

Tumor Cell Biology: Apoptosis and Senescence

P2-04-01  Extracellular PAI-1 - t-PA – IGFBP3 Cascade Mediates Chemotherapy-Induced Senescence of Breast Cancer Cells
Shio Y, Elzi DJ, Song M, Hakala K, Weitstraub ST. The University of Texas Health Science Center, San Antonio, TX.

P2-04-02  Identification of a Novel Glycocalyxtransferase-Like Gene as an Autophagic Inducer in Human Mammary Carcinoma Cells Via Down Regulation of BCL-2

Tumor Cell Biology: Angiogenesis

P2-05-01  Acquired Tamoxifen Resistance Promotes Angiogenic Responses in ER+ Breast Cancer Cells
Hayes E, Smith C, Nicholson RI, Gee J, Hiscox S. Cardiff University, Wales, United Kingdom.
P2-05-02  PGC1, Peroxisome Proliferator Activated Receptor-gamma (PPAR-gamma) Coactivator-1, Is Necessary in PPAR-gamma Modulated Angiogenesis
Jiang WG, Li X, Mansel RE. Cardiff University School of Medicine, Cardiff, Wales, United Kingdom.

P2-05-03  Is Breast Cancer Trypsinase a Novel Anti-Angiogenic Molecular Target?

P2-05-04  Mapping the Specific Gene Families Activated in the Lymphangiogenesis and Vasculogenic Mimicry Exhibited by Inflammatory Breast Cancer
Liu H, Luo AZ, Mu Z, Chu K, Ye Z, Alpbaugh RK, Fernandez S, Boley KM, Jin J, Wright MC, Cristofanilli M, Robertson FM. The University of Texas, MD Anderson Cancer Center, Houston, TX, Fox Chase Cancer Center, Philadelphia, PA.

P2-05-05  Receptor-Like Protein Tyrosine Phosphatase Kappa (PTPRK) and Its Biological Role in Angiogenesis
Sun P-H, Ye L, Mason MD, Jiang WG. Cardiff University School of Medicine, Cardiff, United Kingdom.

P2-05-06  Role of Repulsive Guidance Molecule b (RGMb) in HGF Mediated Angiogenesis
Sanders AJ, Ye L, Mason MD, Jiang WG. Cardiff University School of Medicine, Cardiff, United Kingdom.

P2-05-07  Combined Antiangiogenic and Anti-Estrogen Therapy in Breast Cancer. Molecular Mechanisms Involved
de la Haba J, Berciano M, Cañabate M, Porras I, Valverde A, Cañas A, Rodríguez A, Aranda E. Hospital Universitario Reina Sofia, Institute for Biomedical Research Maimonides IMIBIC, Cordoba, CO, Spain; Hospital General Universitario, Ciudad Real, CR, Spain.

P2-05-08  Expression of VE-Statin/egfl7 in Breast Cancer
Lauridant-Philippin G, Baranelli M-C, Fourrier C, Samson C, Soncin F, Bonnette J. Centre Oscar Lambret, Lille, France; Institut de Biologie de Lille - CNRS UMR 8161, Lille, France; Université Lille Nord de France, Lille, France.

P2-05-09  Secondary Inflammatory Breast Cancer: A Possible Model for Post-Surgical Dissemination of Cancer
Hashmi S, Zolfaghari L, Levine PH. George Washington University, Washington, DC.

P2-05-10  Brain-Derived Neurotrophic Factor, BDNF, and Its Biological Impact on Vascular Endothelial Cells
Jiang WG, Ye L, Yang X, Mansel RE. Cardiff University School of Medicine, Cardiff, Wales, United Kingdom; Capital Medical University, Beijing, China.

Tumor Cell Biology: Genetics – Somatic Changes

P2-06-01  Genomic Instability in Breast Cancers from Atomic Bomb Survivors: An Analysis of Microarray-Comparative Genomic Hybridization with Old Archived Tissues
Oikawa M, Yoshura K-I, Kondo H, Miura S, Kurashige T, Nagayasu T, Yano H, Nakashima M. Atomic Bomb Disease Institute, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan; Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan.

P2-06-02  FOXO3a Genotype Predicts Age of Breast Cancer Onset and Correlates with Lymph Node Involvement

Grizzle WE, Steg AD, He Q, Steciuk MR, Byun-Parker S, Johnson MR, Grunda JM. University of Alabama at Birmingham, Birmingham, AL; Tuskegee University, Tuskegee, AL.

P2-06-04  Phosphatidylinositol-3-Kinase Pathway Mutations Are Common in Breast Columnar Cell Lesions
Truxell ML, Brunner AL, Montgomery K, Zhu SX, Neff T, Warrick A, Beadling C, Corless CL, West RB. Oregon Health & Science University, Portland, OR; Stanford University, Stanford, CA.

Tumor Cell Biology: Genetics – Germline Changes

P2-07-01  Copy Number Variants in Early-Onset-Breast Cancer
Ludwig S, Ivanovich J, Graubert TA. Goodfellow PJ. Washington University in St.Louis, SOM, St. Louis, MO.

P2-07-02  Germline Genetic Variants Disturbing the Let-7/LIN28 Double-Negative Feedback Loop Alter Breast Cancer Susceptibility
Yu K-D, Shao Z-M, Chen A-X. Fudan University Shanghai Cancer Center, Shanghai, China.

P2-07-03  Collective Evidence Suggests Neutrality for BRCA1 V1687i, a Novel Sequence Variant in the Conserved THO Motif of the First BRCT Repeat
Cortesi L, De Nicolai A, Medic V, Marino M, Turchetti D, Pradella LM, Rossi G, Parisini E, Federico M. University of Modena and Reggio Emilia, Modena, Italy; Dana-Farber Cancer Institute, Boston, MA; Harvard Medical School, Boston, MA; University of Bologna, Bologna, Italy; Italian Institute of Technology, Milan, Italy.

Detection and Diagnosis – Imaging and Screening: Breast Imaging – MRI

P2-08-01  Impact of Aromatase Inhibitors on Background Parenchymal Enhancement and Amount of Fibroglandular Tissue on Breast MRI

P2-08-02  Magnetic Resonance Imaging as a Predictor of Pathologic Response in Patients Treated with Neoadjuvant Systemic Treatment for Operable Breast Cancer (TCRC 017)
De los Santos J, Cantor A, Mcguire K, Golshan M, Merci-Bemstam F, Horton J, Nanda R, Amos K, Forero A, Hudis C, Meszoely I, Hwang S. Hospital General Universitario, Ciudad Real, CR, Spain; University of North Carolina Chapel Hill, Chapel Hill, NC; Dana-Farber Cancer Institute, Boston, MA; Duke University Medical Center, Durham, NC; Memorial Sloan Kettering Cancer Center, New York, NY; University of Pittsburgh Medical Center, Pittsburgh, PA; MD Anderson Cancer Center, Houston, TX; Vanderbilt University, Nashville, TN; University of Chicago, Chicago, IL; University of California at San Francisco, San Francisco, CA.

P2-08-03  Quantitative MRI for Noninvasive Prediction of Prognostic Markers in Breast Cancer
Parisian S, Sun R, Kurland BF, Rahbar H, Allison KH, Specht JM, DeMartini WB, Lehrman CD, Partridge SC. University of Washington, Seattle, WA; Fred Hutchinson Cancer Research Center, Seattle, WA.

P2-08-04  Computer-Derived Breast MRI Features Have Complementary Value for Preoperative Selection of Systemic Drug Therapy in Node-Negative Stage-I/II Breast Cancer Patients
Ghilijus KGA, Pengel KE, Dimitriev I, Paape A, Rutgers EEl; Wesseling J, Loo CE. The University Medical Center Utrecht, Utrecht, Netherlands; The Netherlands Cancer Institute/Antoni van Leeuwenhoek Hospital, Amsterdam, Netherlands.
P2-08-05  Use of Dynamic Contrast-Enhanced MR Imaging To Predict Pathological Response in Primary Breast Cancer

P2-08-06  Improved Spatial Resolution Diffusion-Weighted Imaging for Characterizing Tumors and Treatment Response in Patients with Invasive Breast Cancer

P2-08-07  Ex-Vivo High Resolution MRI of Sentinel Lymph Nodes Following Subcutaneous Injection of Superparamagnetic Iron Oxide Nanoparticles

P2-08-08  Benefit of Preoperative Breast MRI in Patients with Newly Diagnosed Breast Cancer
Miesbauer M, Sheu H, Schiessingegr A, Kirchweger B, Waldenberger P. St. Vincent Hospital, Linz, Austria.

P2-08-09  The Impact of Preoperative Breast MRI in Newly Diagnosed Breast Cancer. A Prospective Study of Treatment Outcome in Patients Selected for Breast-Conserving Surgery in a Norwegian Multidisciplinary Breast Cancer Clinic
Robben T, Rodegerdts E, Bachmann H, Funder V. Department of Surgery, Nordland Hospital, Bodø, Norway; Department of Radiology, Nordland Hospital, Bodø, Norway; Faculty of Health Sciences, University of Tromsø, Tromsø, Norway.

P2-08-10  Withdrawn

P2-08-11  Correlation of Mammography, Ultrasound and MRI in Patients with Nipple Discharge
Deshmane VH, Mulan S, Lodh N, Pungavkar S, Bhaduri A, Deshpande R, Madiwale C. P. D. Hinduja National Hospital, Mumbai, Maharashtra, India; Nanavati Hospital, Mumbai, Maharashtra, India.

P2-08-12  Additional Lesion Found in Preoperative Breast MRI; Is Routine Use Justified?


Detection and Diagnosis – Imaging and Screening: Molecular, Functional, and Novel Imaging

P2-09-01  First-in-Human Whole-Body HER2-Receptor Mapping Using Affibody Molecular Imaging

P2-09-02  Predicting Response to Bevacizumab in Primary Breast Cancer Using 18F-Fluorothymidine (FLT) and 18F-Misonidazole (MISO) Positron Emission/Computed Tomography (PET/CT) as Imaging Biomarkers

P2-09-03  Breast Cancer Detection Using a Novel Functional Imaging Device Equipped with Multiparametric Computer Analysis
Sella T, Sklar-Levy M, Maya C, Rozen M, Tanim A, Maisa S, Libson E, Ishaky D. Hadassah Hebrew University Medical Center, Jerusalem, Israel; Chaim Sheba Medical Center, Tel Hashomer, Israel; Rabin Medical Center, Petach Tikva, Israel; Assuta Medical Center, Tel-Aviv, Israel; Kaplan Medical Center, Rehovot, Israel; Meir Medical Center, Kfar Sava, Israel; Real Imaging Ltd., Airport City, Israel.

P2-09-04  Near Infra Red Quantum Dots as Novel Probes for Sentinel Lymph Node Biopsy

P2-09-05  Molecular Imaging of Hedgehog Induced Epithelial-Stromal Interactions
Woodward W, Sims-Mourtada J, Yang DJ, Twerorswka I, Larson R, Smith D, Mourtada F. RadioMedix, Inc, Houston, TX; The University of Texas M.D. Anderson Cancer Center, Houston, TX.

P2-09-06  Relevance of Breast Cancer Subtypes in Response Monitoring with 18F-FDG PET/CT during Neoadjuvant Chemotherapy

P2-09-07  Metabolic Response by FDG-PET in Patients (pts) Receiving Trastuzumab (T) and Lapatinib (L) for HER2+ Metastatic Breast Cancer (MBC): Correlative Analysis of TBCRC 003
Yap JT, Locascio T, Najita JS, Mayer IA, Hobday TJ, Falkson CI, Dees EC, Gelman RS, Rimawi MF, Nanda R, Berkowitz J, Franchetti Y, Wolff AC, Winer EP, Lin N, Van den Abbeele AD. Dana-Farber Cancer Institute, Boston, MA; Vanderbilt-Ingram Cancer Center, Nashville, TN; Mayo Clinic, Rochester, MN; University of Alabama, Birmingham, AL; University of North Carolina at Chapel Hill, Chapel Hill, NC; Baylor College of Medicine, Houston, TX; University of Chicago, Chicago, IL; Johns Hopkins Kimmel Cancer Center, Baltimore, MD.

P2-09-08  FDG-PET in the Staging of the Axilla in Women with Breast Cancer Treated with Primary Chemotherapy

P2-09-09  Dynamic FDG PET and DCE-MRI To Assess Tumor Metabolism and Blood Flow in Response to Neoadjuvant Sunitinib and Paclitaxel Followed by AC + G-CSF in Patients with Locally-Advanced (LABC) and/or Inflammatory Breast Cancer (IBC)
Schecht JM, Kurland BF, Dunnwald LK, Doot RK, Eun JK, Schubert EK, Partridge SC, Ellis GK, Gadi VK, Gralow JR, Linden HM, Rodler ET, Mankoff DA. University of Washington, Seattle, WA; Fred Hutchinson Cancer Research Center, Seattle, WA; Seattle Cancer Care Alliance, Seattle, WA.


P2-09-12 High Resolution Diffusion MRI Characterizes Tumor Stromal Boundaries McLaughlin RL, Newitt DC, Wilmes LS, Sinha S, Wisner DJ, Hylton NM. UC Berkeley and UCSF, Berkeley and San Francisco, CA; UCSF, San Francisco, CA.

P2-09-13 The Value of FDG PET/CT in Screening Detected Breast Cancer Patients Koo HR, Moon WK, Cho N, Chang JM, Kang KW, Yi A. Seoul National University Hospital, Seoul, Korea.

P2-09-14 Evaluation of Angiogenesis Using Synchrotron Radiation in Xenograft Mouse Models of Breast Cancer Gu S-m, Zhang X, Li R-m, Jin W, Shen Z-z, Shao Z-m, Xu X-m, Wu J. Cancer Hospital/Institute, Fudan University, Shanghai, China; Shanghai Jiao Tong University, Shanghai, China.


Detection and Diagnosis – Imaging and Screening: Breast Imaging – Other Methods

P2-10-01 Extracellular Matrix Stiffness and Mammographic Density in the Human Breast Acerbi L, Au A, Chen Y-y, Hwang S, Weaver V. Center for Bioengineering and Tissue Regeneration, University of California San Francisco, San Francisco, CA; University of California San Francisco, San Francisco, CA; Eli and Edythe Broad Center of Regeneration Medicine and Stem Cell Research, and Helen Diller Family Comprehensive Cancer Center, UCSF, San Francisco, CA.

P2-10-02 Assessment of Tumor Response to Neoadjuvant Chemotherapy Using Ultrasound-Guided Near Infrared Light Zhu Q, DeFusco P, Tannenbaum S, Ricci A, Cronin E, Hegde P, Kane M, Tavakoli B, Xu Y. University of Connecticut; Storrs, CT; Hartford Hospital; Hartford, CT; University of Connecticut Health Center, Farmington, CT.

P2-10-03 Non-Invasive In Vivo Characterization of Cancer-Cell Proliferation & Angiogenesis in Cancer-Cell-Surrounding Stromal Microenvironment In-Vivo Using Diffuse Optical Tomography Chung SH, Feldman M, Choe R, Martinez D, Yodh AG. University of Pennsylvania; Hospital of the University of Pennsylvania; The Children's Hospital of Philadelphia, University of Rochester.

P2-10-04 3D Mapping of Total Choline in Human Breast Cancer Using High-Speed MR Spectroscopic Imaging at 3T: A Feasibility Study Posse S, Royce M, Dayao ZR, Zhang T, Zhao C, Sillerud L, Lopez S, Casey L, Eberhardt SC, Lomo L, Lee S-J, Rosenberg R, Rainey A, Bolan P. University of New Mexico School of Medicine, Albuquerque, NM; University of New Mexico Cancer Center, Albuquerque, NM; New Mexico Cancer Center, Albuquerque, NM; University of Minnesota, Minneapolis, MN.


P2-10-06 Prospective Ultrasonographic Prediction of Sentinel Lymph Node Metastasis by Real-Time Virtual Sonography Constructed with Three-Dimensional Computed Tomography-Lymphography in Breast Cancer Patients Yamamoto S, Maeda N, Yoshimura K, Oka M. Yamaguchi University Graduate School of Medicine, Ube, Yamaguchi, Japan.

P2-10-07 Triple-Negative Breast Cancer May Have Ultrasonographic Features Mimicking Nonmalignant Lymph Nodes Wojcinski S, Farrokh A, Schmidt J, Hillemanns P, Degenhardt F. Franziskus Hospital, Bielefeld, Germany, Hannover Medical School, Hannover, Germany.

P2-10-08 Electrical Impedance Imaging Characteristics of Nodular and Edematous-Infiltrative Forms of Breast Cancer Korotkova M, Karpov A, Bulatov A, Frzyuk A. Clinical Hospital 9, Yaroslavl, Russian Federation.

P2-10-09 Detecting Breast Cancer with Dynamic Diffuse Optical Tomographic Imaging Flexman ML, Kim HK, Lim E, Desperito E, Barbour RL, Hershman DL, Hielscher AH. Columbia University, New York, NY; State University of New York, Brooklyn, NY.


Prognosis/Response Predictions: Prognostic Factors and Biomarkers – Preclinical


P2-11-02 Brain-Derived Neurotrophic Factor Expression Is Associated with Poor Prognosis in Human Breast Cancer Patani N, Jiang WG, Mokbel K. The London Breast Institute, The Princess Grace Hospital, London, United Kingdom; University Department of Surgery, Cardiff University School of Medicine, Cardiff, United Kingdom; Brunel Institute of Cancer Genetics and Pharmacogenomics, Brunel University, London, United Kingdom.

P2-11-03 High Expression of CX3CL1 by Tumor Cells Correlates with a Good Prognosis and Increased Infiltrating CD8+ T Cells, Natural Killer Cells, and Dendritic Cells in Breast Carcinoma Lee JS, Park MH, Yoon JH. Chonnam National University Medical School, Gwangju, Republic of Korea.

P2-11-04 TMEPAI Is a Feedback Regulator of TGF-β Signaling during Breast Cancer Progression Singhia PK, Pandeswarra S, De La Chapa JJ, Venkatachalam MA, Saikumar P. UT Health Science Center at San Antonio, San Antonio, TX.
P2-11-05 Stromal Matrix Metalloproteinase-14 Expression Correlates with the Grade and Biological Behavior of Mammary Phyllodes Tumors
Lee JS, Park MH, Yoon JH. Chonnam National University Medical School, Gwangju, Republic of Korea.

P2-11-06 PTEN Loss in Asian Triple Negative Breast Cancer Is a Frequent Event Associated with Basal Markers, Tumour Grade and Younger Age of Onset
Dean S, Rhodes A, Holly J, Perks C, Loci L-AM, Mun KS, Taib NA, Yip CH. University of the West of England, Bristol, United Kingdom; University of Bristol, Bristol, United Kingdom; University of Malaya, Kuala Lumpur, Malaysia.

P2-11-07 Expression of Selected Predictive Markers in African American Women with Atypical Hyperplasia of the Breast
Bandopadhyay S, Cote M, Visscher DW, Rutberbus J, Albashiti B, Alosl B, Frost MH, Hartmann LC, Fehmi RA. Wayne State University/KCI, Detroit, MI; Wayne State University/KCI, Detroit, MI; Mayo Clinic Cancer Center, Rochester, MN.

P2-11-08 Clinical Value of Combination Assay for Quantitative Determination of Cancer Biomarkers C2p and uPA/PAI-1 for Disease Recurrence Prediction of Early Breast Cancer Patients
Schmitt M, Kiechle M, Schwarz-Boeger U, Langer R, Nakayama S, Matsuhash T, Ishihata H. Technical University of Munich, Munich, Germany; Sysmex Corporation, Kobe, Japan.

P2-11-09 EGFR Overexpression in Triple Negative Breast Cancer (TNBC) and Its Association with the Prognosis
Liu W, Zhang L, Ma K, Han B, Li S, Xu G, Fan Z, Liu N, Shi A. The First Hospital of Jilin University, Changchun, Jilin, China; The 208 Hospital of People’s Liberation Army, Changchun, Jilin, China; The Central Hospital of Siping, Siping, Jilin, China; The Second Hospital of Jilin University, Changchun, Jilin, China.

P2-11-10 Low Toll-Like Receptor 9 Expression Is Required for the Aggressive Behavior of Triple Negative Breast Cancer Cells in Hypoxia
Selander KS, Tuomela J, Sandholm J, Kanihalta P, Pressey C, Ilvesar J, Vuopala K, Kaupilla JH, Kaupilla S, Harris KW, Graves D, Jurkasla-Vuoren J. University of Alabama at Birmingham, Birmingham, AL; Oulu University Hospital, Oulu, Finland; University of Oulu, Oulu, Finland.

P2-11-11 IGBP-2 Ratio Confers Resistance to IGF Targeting and Correlates with Increased Invasion and Poor Outcome in Breast Tumors
Becker MA, Hou X, Harrington SC, Carbini JM, Gottardis MM, Haluska F, Mayo Clinic, Rochester, MN; Bristol Myers Squibb Pharmaceutical Research Institute, Princeton, NJ.

P2-12-03 A Prospective Study of the Prognostic Implications of Being a BRCA1 Carrier for Young Onset Breast Cancer Patients
Erick DM, Diet L, Gemy SM, Altman D, Copson ER, Simononds PD, Durcan L, Ward D. University of Southampton, Southampton, Hampshire, United Kingdom; Oxford University, Oxford, United Kingdom; Southampton University Hospitals NHS Trust, Southampton, Hampshire, United Kingdom; Salisbury District Hospital, Salisbury, Wiltshire, United Kingdom.

P2-12-04 RACGAP1 mRNA Assay Outperforms Ki-67 as a Proliferation Marker in the FinHer Study Cohort
Wirtz RM, Leinonen M, Bono P, Isola J, Kellokumpu-Lehtinen P-L, Kataja V, Turpeenniemi-Hujanen T, Jyrkkio S, Huang W, Eids T, Joensuu H. STRATIFYER Molecular Pathology GmbH, Cologne, Germany; Pharma, Finland; Helsinki University Central Hospital and University of Helsinki, Finland; University of Tampere and Tampere University Hospital, Finland; Tampere University Hospital, Finland; Kuopio University Hospital, Finland; Oulu University Hospital, Finland; Turku University Hospital, Finland; Monogram Biosciences, Inc.; Institute of Pathology at the St-Elisabeth-Hospital, Germany.

P2-12-05 Correlation between Quantitative HER2 Protein Expression and Risk of Brain Metastases in HER2-Positive Advanced Breast Cancer Patients Receiving Trastuzumab-Containing Therapy
Duchnowska R, Bianetti W, Szostakiewicz B, Spierde J, Piette F, Haddad M, Paquet A, Lie Y, Czartoryska-Arlukowicz B, Wysocz P, Jankowski T, Radecka B, Fozszycka-Kloda M, Liwinski M, Delska S, Weidler J, Huang W, Buyse M, Bates M, Jassem J. Military Institute of Medicine, Warsaw, Poland; Medical University of Gdansk, Gdansk, Poland; Monogram Biosciences, South San Francisco, CA; International Drug Development Institute, Louvain-la-Neuve, Belgium; Bialystok Oncology Center, Bialystok, Poland; Great Poland Cancer Center, Poznan, Poland; Lublin Oncology Center, Lublin, Poland; Opole Oncology Center, Opole, Poland; West Pomeranian Oncology Center, Szczecin, Poland; Poznan University of Medical Sciences, Poznan, Poland; Regional Cancer Center, Lodz, Poland; Ceghein, Sunyvalle, CA.

P2-12-06 Nomogram To Predict Subsequent Bone Metastasis in Patients with Non Metastatic Breast Carcinomas
Louqui R, Delpech Y, Roussier R, Gispon J, Hui L, Barranger E, Puzaite L, Uzan S, Hortobagyi GN, Courant C, Ibrahim NK. Lariboisiere Hospital, AP-H, Paris, France, Metropolitan; Tenon Hospital, AP-H, Paris, France, Metropolitan; The University of Texas M.D. Anderson Cancer Center, Houston, TX; Georges Francois Leclerc Cancer Center, Dijon, France, Metropolitan.

P2-12-07 Pooled Analysis of Outcomes of T1a/BNO, HER2-Amplified Breast Cancer
Amir E, Seruga B, Ocan A, Carlsson L, Bedard P. Princess Margaret Hospital, Toronto, Canada; Institute of Oncology Ljubljana, Ljubljana, Slovenia; Albacete University Hospital, Albacete, Spain.

P2-12-08 Bcl-2 as a Prognostic Marker in Breast Cancer Patients Receiving Endocrine Therapy
Larsen MS, Bjerre KD, Laenholm A-V, Giobbie-Hurder A, Ejertsen B, Lykkefeldt AE, Rasmussen BB. Herlev Hospital, Herlev, Denmark; Rigshospitalet, Copenhagen, Denmark; Dana-Farber Cancer Institute, Boston, MA, Danish Cancer Society, Inst of Cancer Biology, Copenhagen, Denmark.

P2-12-09 Prediction of Risk of Recurrence (RRR) after 5-Years of Follow-Up by Ki-67 and IHC4: A TransATAC Study
Pineda S, Cuzick J, Salter J, Zabaglo L, Howell A, Buzdar A, Forbes JF, Dowsett M. Queen Mary University of London, London, United Kingdom; Royal Marsden Hospital and Breakthrough Breast Cancer Centre, London, United Kingdom; Christie Hospital, Manchester, United Kingdom; University of Texas, Texas; University of Newcastle, Newcastle, NSW, Australia.

www.aacrjournals.org Cancer Res; 71(24 Suppl.) December 15, 2011
P2-12-10 Low TCR Diversity (Divpenia) Is a Prognosis Factor of Overall Survival in Metastatic Breast Cancer

P2-12-11 Clinical Relevance of a IL-8/B-Cell Gene Signature Identified from Trigle Negative Breast Cancer (TNBC) in Intrinsic Breast Cancer Subtypes
Rodr G, Holtrich U, Ruckhaeberle E, Radossa J, Juhasz-Boess I, Solomayer EF, Kaufmann M, Karm T, Saarland University, Homburg, Germany; Goethe-University, Frankfurt, Germany.

P2-12-12 Prognostic Utility of Breast Cancer Index for Late Relapse in Patients with Early Stage Breast Cancer

P2-12-13 Topoisomerase 2 alpha (TOP2A) RNA Expression Provides Prognostic Information in Hormone Receptor Positive Breast Cancer That Is Complementary to a Simulated Algorithm for Recurrence Score
Sparano JA, Goldstein LJ, Davidson NE, Sledge, Jr GW, Gray R. Albert Einstein College of Medicine, Montefiore Medical Center, Bronx, NY; Fox Chase Cancer Center, Philadelphia, PA; University of Pittsburgh, Pittsburgh, PA; Indiana University, Indianapolis, IN; Eastern Cooperative Oncology Group, Brookline, MA.

P2-12-14 Prognostic Value of HER2 Positivity and Negative Hormonal Status in Patients with Small Tumor (<1cm) and Node-Negative Breast Cancer
Meattini I, Livi S, Saieva C, Agresti B, Scotti V, Nori J, Sanchez LJ, Vezzosi V, Bianchi S, Cataliotti L, Biti G. Florence University, Florence, Italy; Cancer Prevention and Research Institute, Florence, Italy.

P2-12-15 GAINS: A Breast Cancer Prognostic Index Utilizing Lymph Node Ratio
Doranj JI, Wall D, Newell J, Blamey RW, Sweeney KJ, Ball G, Kerm M. National University of Ireland, Galway, Ireland; Breast Institute, Nottingham City Hospital, Nottingham, United Kingdom; Nottingham Trent University, Nottingham, United Kingdom.

P2-12-16 HER2 Expression Is the Major Risk Factor for Recurrence in pT1a-b,N0 Breast Cancer: A French Regional Population-Based Study of 671 Patients

P2-12-17 Prospective Evaluation of the Conversion Rate of HER2, ER and PR between Primary Tumours and Corresponding Metastases.
CONVERTER/GEICAM 2009-03 Study
Martinez de Dueñas E, Lluch A, Guerrero A, Chacon JJ, Perez R, Antolin S, Blancas I, Ferrer-Lozano J, Burgues O, Lopez A, Gonzalez-Angulo AM. Hospital Provincial de Castellón, Castellón, Spain; Hospital Clínico Universitario de Valencia, Valencia, Spain; Instituto Valenciano de Oncología, Valencia, Spain; Hospital Virgen de la Salud de Toledo, Toledo, Spain; Hospital Universitario Quiron, Madrid, Spain; Complejo Hospitalario Universitario A Coruña, A Coruña, Spain; Hospital Clínico San Cecilio de Granada, Granada, Spain; MD Anderson Cancer Center, Houston, TX.

P2-12-18 A Prognostic Model Based on Node Status, Cathespin-D and Ki-67 Predict the Outcome of Patients Failing To Achieve Pathological Complete Response after Anthracycline-Based Neoadjuvant Chemotherapy for Breast Cancer
Chen S, Chen C, Yu K, Shao Z. Shanghai Cancer Hospital, Fudan University, Shanghai, China.

P2-12-19 Nomogram To Predict Recurrence and To Avoid Unnecessary Adjuvant Chemotherapy Based on Ki67 Index and ER Status in Hormone Receptor (HR)-Positive Breast Cancers with Low Number of Nodal Metastases (≤3) (NCT01273415)

P2-12-20 Adjuvant! Online Is Overoptimistic in Predicting Survival of Asian Breast Cancer Patients
Boho Pathy N, Yip CH, Hartman M, Saxena N, Taib NA, Bulgiba AM, van der Graaf Y, Verkooijen HM. University of Malaya; University Medical Center Utrecht; Ministry of Health Malaysia; National University of Singapore; Karolinska Institutet.

P2-12-21 Impact of Recent Parity on Histopathological Tumor Features and Outcome of Young Women with Breast Cancer
Nagatsuka KM, Shimizu C, Tsuda H, Saji S, Hojo T, Sugano K, Fujivara Y. National Cancer Center Hospital; Saitama Medical University International Medical Center, Tochigi Cancer Center Research Institute.

P2-12-22 A Prognostic Index Composed of Progesterone Receptor Status, Tumour Size and 5-phase Fraction, Predicts Survival in Node-Negative Breast Cancer Patients in a Large Multicentre Prospective Cohort Study
Klintman M, Nilsson F, Bendahl P-O, Fernö M, Liljegren G, Emdin S, Malmström P. Clinical Sciences, Lund University and Skane University Hospital, Lund, Sweden; University of Umeå and Umeå University Hospital, Umeå, Sweden; orebro University Hospital, orebro, Sweden.

P2-12-23 How Should We Assess Tumour Size (T Stage) in Patients with Multicentric/Multifocal Breast Cancer? Results from the NCIC CTG MA.5 Randomized Trial of CEF vs. CMF in Pre-Menopausal Women with Node Positive Breast Cancer
Bouganim N, Dong B, Hilton JF, Chapman J-AW, Arnaout A, O’Malley F, Nielsen T, Gelmon K, Yershalmi R, Levine M, Bramwell V, Whelan T, Pritchard K, Shepherd L, Clemons M. The Ottawa Cancer Center, Ottawa, ON, Canada; Queens University, Kingston, ON, Canada; The Ottawa Hospital, Ottawa, ON, Canada; Mount Sinai Hospital, University of Toronto, Toronto, ON, Canada; Vancouver Hospital and Health Sciences Center, Vancouver, BC, Canada; BC Cancer Agency-University of British Columbia, Vancouver, BC, Canada; Juravinski Cancer Center-McMaster University, Hamilton, ON, Canada; Tom Baker Cancer Center-University of Calgary, Calgary, AB, Canada; Odette Cancer Center-University of Toronto, Toronto, ON, Canada.

P2-12-24 Resumption or Persistence of Menstruation after Cytotoxic Chemotherapy Is a Poor Prognostic Factor for Disease Free Survival in Premenopausal Patients with Early Breast Cancer
Park IH, Han HS, Lee KS, Kang HS, Lee S, Kim SW, Jung SY, Shin KH, Ro J. National Cancer Center, Goyang, Korea; Chungbuk National University, Korea.

P2-12-25 Blinded Multi-Site Validation of a Pathology-Based Prognostic Marker Profile for Operable Hormone Receptor-Positive Breast Cancer
Linke SP, Bremer TM, Man AK, Bloom KJ. Prediction Sciences, La Jolla, CA; Clarient, Inc., Aliso Viejo, CA; Seattle Breast Pathology Consultants, Seattle, WA.
P2-12-26 Impact of the Recurrence Score on Adjuvant Decision-Making in ER-Positive Early Breast Cancer - Results of a Large Prospective Multicenter Decision Impact Study in Node Negative and Node Positive Disease

Rezai M, Eiermann W, Kümmel S, Kühn T, Warm M, Friedrichs K, Schneweiss A, Markmann S, Eggemann H, Hilfrich J, Jackisch C, Witzel I, Eidtmann H, Kaufmann M, Blohmer JU. Lusenhanzenhaus, Düsseldorf, Germany; Rotkreuzklinikum, München, Germany; Klinikum Essen-Mitte, Essen, Germany; Klinikum Esslingen, Esslingen, Germany; Krankenhaus Holweide, Köln, Germany; Brustzentrum, Hamburg, Germany; Universitätsklinikum, Heidelberg, Germany; Universitätsklinikum Rostock, Germany; Universitätsklinikum Magdeburg, Germany; Ellenriedeklinik, Hannover, Germany; Klinikum Offenbach, Offenbach, Germany; Universitätsklinikum Eppendorf, Hamburg, Germany; Universitätsklinikum Kiel, Germany; Universitätsklinikum Frankfurt, Germany; Sankt Gertraudenh-Krankenhaus, Berlin, Germany.

P2-12-27 Simply Adding Together the Diameters of Tumor Foci in Patients with Multicentric or Multifocal Disease Does Not Add Any Additional Prognostic Information: An Analysis from NCIC CTG MA.12 Randomized Placebo-Controlled Trial of Tamoxifen after Adjuvant Chemotherapy in Pre-Menopausal Women with Early Breast Cancer

Hilton JF, Dong B, Bouganim N, Chapman J-AW, Arnaout A, O’Malley F, Nielsen T, Gelmon K, Yerushalmi R, Levine M, Bramwell V, Whelan T, Pritchard K, Shepherd L, Clemons M. Queens University, Kingston, ON, Canada; The Ottawa Hospital, University of Ottawa, Ottawa, ON, Canada; Mt. Sinai Hospital, University of Toronto, Toronto, ON, Canada; Vancouver Hospital and Health Sciences Centre, University of British Columbia, Vancouver, BC, Canada; BC Cancer Agency, University of British Columbia, Vancouver, BC, Canada; Juravinski Cancer Centre, McMaster University, Hamilton, ON, Canada; Tom Baker Cancer Centre, University of Calgary, AB, Canada; Odette Cancer Centre, University of Toronto, Toronto, ON, Canada.

P2-12-28 Studies of a Malignancy-Associated Protein, Osteopontin, in NCIC CTG MA.14, a Randomized Trial of Tamoxifen Versus Combined Tamoxifen and Octreotide LAR in Adjuvant Treatment of Women with Early Breast Cancer


P2-12-30 Pre-Operative Haematological Markers and Prognosis in Early Breast Cancer

Cordiner RL, Mansell J, Obono C, Angerson WJ, Lannigan A, McMillan D, Wilson CR, Doughty JC. Western Infirmary, Glasgow, United Kingdom; Wishaw General Hospital, Wishaw, United Kingdom.

P2-12-31 Moderate Immunohistochemical Expression of HER2 (2+) without HER2 Gene-Amplification Is a Negative Prognostic Factor in Early Breast Cancer

Rossi V, Sarotto I, Maggiarotto F, Tomasi Cont N, Redana S, Aglietta M, Ponzone R, Montemurro F. Institute for Cancer Research and Treatment IRCC, Candidolo, Turin, Italy.

P2-12-32 Association between Progranulin (GP88) Expression and Recurrence Risk for Breast Cancer Patients with Estrogen Receptor Positive Invasive Ductal Carcinoma

Serreno G, Hawkins DM, Ioffe O, Bejarano P, Phillips JT, Head JE, Elliott RL, Godwin AK, Weaver J, Yue B. A&G Pharmaceutical Inc, Columbia, MD; University of Minnesota, Minneapolis, MN; University of Maryland, Baltimore, MD; University of Miami, Miami, FL; EHE Breast Cancer Research and Treatment Center, Baton Rouge, LA; University of Kansas Medical Center, Kansas City, KS; Fox Chase Cancer Center, Philadelphia, PA.

P2-12-33 Withdrawn

Epidemiology, Risk, and Prevention: Familial Breast Cancer – Genetic Testing

P2-13-01 Gene Profiling of Whole Blood May Identify Patients with BRCA Mutations

Mina LA, Gokmen-Polar Y, Goswami C, Storniolo AM, Li L, Badse V, Sledge GW. Indiana University School of Medicine, Indianapolis, IN.

P2-13-02 Parent of Origin of BRCA Mutation May Determine Age at Breast Cancer Diagnosis

Shapira I, Budman DR, Akerman M, Weiselberg L, Vinciguerra V, D’Olimpio J, Devoe C, Cheng K, Donahue L, John V, Cohen S. Hofstra North Shore UJ School of Medicine, Lake Success, NY; Feinstein Institute for Medical Research, Manhasset, NY.


Beattie MS, Ganschow P, Gabram-Mendola S, Wilson A, Joseph G, Lee R, Loranger K, Stanslaw C, Seelaus C, Farrell R, Trim J, DelPozzo S, Luce J. University of California, San Francisco, CA; San Francisco General Hospital, San Francisco, CA; Stroger Hospital of Cook County, Chicago, IL; Rush University Medical Center, Chicago, IL; Emory University, Atlanta, GA; Grady Memorial Hospital, Atlanta, GA.

P2-13-04 Testing Women with Invasive Lobular Breast Cancer for BRCA Mutations

Turco DL, Elsayegh N, Litton J, Hortobagyi GN, Arun B. MD Anderson Cancer Center, Houston, TX.

P2-13-05 Breast Cancer, BRCA Mutations and Attitudes Regarding Pregnancy and Preimplantation Genetic Diagnosis

Litton JK, Ezel C, Jackson MA, Muse JJ, Turco D, Schover LR, ThanEUart RL, Mattar D, Lu KH, Hortobagyi GN, Arun BK. The University of Texas MD Anderson Cancer Center, Houston, TX.

P2-13-06 Withdrawn

P2-13-07 Prevalence of Germline BRCA1 and BRCA2 Deletions Mutations in Brazilian Patients with High-Risk of Breast and Ovarian Cancer

Diz MDPE, Escobar KA, Guindalini CSC, Pasini FS, Snitkovski IM, Maistro S, Hof PMG, Federico MHH. Instituto do Cancer do Estado de Sao Paulo, Sao Paulo, Brazil; Faculdade de Medicina da Universidade de Sao Paulo, Sao Paulo, Brazil.

P2-13-08 A Retrospective Study on Surveillance Behaviour and Prophylaxis after Genetic Testing for Hereditary Breast and Ovarian Cancer among High-Risk Chinese Females

Kwong A, Chu ATW. The University of Hong Kong, Hong Kong; The Hong Kong Hereditary Breast Cancer Family Registry, Hong Kong.

P2-13-09 First Statewide Experience with Telephone Delivery of Cancer Genetic Counseling


P2-13-10 Detection, Visualization and High Resolution Physical Mapping of Large Rearrangements by Molecular Combing in the Hereditary Breast Cancer Genes BRCA1 and BRCA2


P2-13-11 Follow Up of BRCA1/2 Carriers: The Spectrum of Cancer Diagnoses in Healthy at Risk Individuals (HTR), and in Cancer Survivors (CS)

P2-13-12 The impact of a directive counseling procedure in families with a BRCA1/2 gene mutation: uptake of predictive genetic testing and general outcome

Epidemiology, Risk, and Prevention: Ethnic/Racial Aspects

P2-14-01 Race, Response to Chemotherapy, and Outcome within Clinical Breast Cancer Subtypes
Tichy JP, Deal AM, Anders CK, Carey LA. UNC, Chapel Hill, NC.

P2-14-02 NCIC CTG MA.27: Clinical Tolerability and Overall Survival of Racial and Ethnic Minority Women on Aromatase Inhibitor Therapy
MoY B, Shepherd LE, Chapman J-AW, Le Maitre A, Gelmon KA, Elliott C, Ingle JN, Goss PE. Massachusetts General Hospital, Boston, MA; National Cancer Institute of Canada Clinical Trials Group, Kingston, ON, Canada; British Columbia Cancer Agency, Vancouver, BC, Canada; Mayo Clinic, Rochester, MN.

P2-14-03 A Comparison of Biologic Differences in Tumors in a Matched Cohort of Hispanic and Caucasian Women with Early-Stage Breast Cancer Using the 21-Gene Recurrence Score Assay
Lim EA, Hershman DL, Greenleese H, Crew KD, Mauer MA, Hibshoosh H, Kalinsky K. Columbia University Medical Center, New York, NY; Mailman School of Public Health, New York, NY.

P2-14-04 The Influence of Demographic, Psychosocial and Emotional Barriers to Screening for Colorectal and Ovarian Cancer among Latina Breast Cancer Survivors
Ramirez AG, Holden AE, San Miguel SL, Galloin KJ. UT Health Science Center at San Antonio, San Antonio, TX.

P2-14-05 Racial Differences in the Use of Adjuvant Chemotherapy for Breast Cancer in a Large Urban Integrated Health System
Simon MS, Lamerato L, Krajenta R, Booza J, Rutertbusch J, Kunz S, Schwartz K. Karmanos Cancer Institute at Wayne State University, Detroit, MI; Henry Ford Health System, Detroit, MI; Wayne State University, Detroit, MI.

P2-14-06 Clinical and Pathologic Characteristics of Haitian Breast Cancer Patients in a Tertiary Care Safety Net Hospital
Loch MM, Ross AA, Rosenberg C, Blanchard RA. Boston University Medical Center, Boston, MA; Boston University, Boston, MA.

P2-14-07 Ethnic Differences in Breast Cancer Molecular Subtypes and Survival Outcomes in a Multi-Ethnic Singaporean Population

P2-14-08 Hormone Receptor Status and Breast Cancer Survival among Hispanic and Non-Hispanic White Women over 10 Years of Follow-Up
Denkhoﬀ SR, Baumgartner KB, Yang D, Pinkston CM, Baumgartner RN. University of Louisville, Louisville, KY.

P2-14-09 Comparison between Spanish and Peruvian Patients with Early Breast Cancer

P2-14-10 Are There Variations in Invasive Tumour Characteristics between Different Ethnic Groups?
Barri B, Lo M, Gandamihardja T, Lewis J, Hogben K. Imperial College Healthcare NHS Trust, Charing Cross Hospital, London, United Kingdom.

P2-15-01 Surgical Patterns of Care after Magnetic Resonance Imaging in the Academic Setting in Patients with Operable Breast Cancer Treated with Neoadjuvant Systemic Therapy: A Secondary Analysis of TBRC 017
De Los Santos J, Cantor A, Mcguire K, Golshan M, Meric-Bernstam F, Horton J, Nanda R, Amos K, Forero A, Hudis C, Meszoely I, Hwang S. University of Alabama at Birmingham, Birmingham, AL; University of North Carolina Chapel Hill, Chapel Hill, NC; Dana-Farber Cancer Institute, Boston, MA; Duke University Medical Center, Durham, NC; Memorial Sloan Kettering Cancer Center, New York, NY; University of Pittsburgh Medical Center, Pittsburgh, PA; MD Anderson Cancer Center, Houston, TX; Vanderbilt University, Nashville, TN; University of Chicago, Chicago, IL; University of California at San Francisco, San Francisco, CA.

P2-15-02 Are We Performing More Mastectomies on Women Diagnosed with Invasive Breast Cancer?
Lo MCI, Hariri B, Gandamihardja T, Lewis J, Hogben K. Imperial College Healthcare NHS Trust, Charing Cross Hospital, London, United Kingdom.

P2-15-03 Nipple-Sparing Mastectomy for Breast Cancer in Japanese Experience: An Assessment from Long Follow-Up Data of Complication and Cancer Control in 806 Patients
Sakurai T, Sakurai T, Inuma T, Jinta E, Suzuma T, Yoshimura G. Wakayama Medical University Kihoku Hospital, Ito, Wakayama, Japan; Sakurai Breast Clinic, Wakayama, Japan; Kiwa Hospital, Japan; Kushimoto Ando Hospital, Japan; Kishiwada Shimin Hospital, Japan.

P2-15-04 Clinical Significance of Resection with Curative Intent for Isolated Pulmonary Metastases from Breast Cancer. Multi-Institutional Study in Japan
Sato N, Ohsumi S, Iwase T, Inaji H, Muzutani M, Nishimura R, Mukai H. Niigata Cancer Center Hospital, Niigata, Japan; National Hospital Organization Shikoku Cancer Center, Matsuyama, Japan; Cancer Institute Hospital, Tokyo, Japan; Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka, Japan; National Hospital Organization Osaka National Hospital, Osaka, Japan; Kumamoto City Hospital, Kumamoto, Japan; National Cancer Center East, Kashiwa, Japan.

P2-15-05 Excision of the Primary Tumour in Patients with Metastatic Breast Cancer - Will E2108 Provide the Deﬁnitive Answer?

P2-15-06 Breast Cancer Liver Metastases - Possibilities and Limits of Surgical Treatment
Narsanska A, Treskova I, Treskova V, Skalicky T, Sutnar A. University Hospital and Medical Faculty Pilsen, Charles University Prague, Pilsen, Czech Republic.

P2-15-07 The Need for Additional Surgeries to Adequately Excise Early Breast Cancers May Have a Negative Impact on Local Recurrence

P2-15-08 New Trends for Surgical Treatment of Large Breast Tumors
Zucca Matthes AG, Vieira RAC, Maki S, Menezes AC, Alvesca MA, Uemura G. Barretos’Cancer Hospital, Barretos, SP, Brazil; School of Medicine of Botucatu - UNESP, Botucatu, SP, Brazil; USP, Sao Paulo, SP, Brazil; University Barão de Mauá, Ribeirão Preto, SP, Brazil.

P2-15-09 Prospective Randomized Comparison of Conventional Instruments and the Harmonic Focus® Device in Breast-Conserving Therapy for Primary Breast Cancer
Bohm D, Kubita A, Lebrecht A, Schmidt M, Kolb H. University Medical Centre, Mainz, Germany.
P2-15-10 Do Patients with Incurable Advanced Breast Tumor and Distant Metastasis Derive Any Benefit from Primary Tumor Resection? 

P2-15-11 Outcomes of Corrective Procedure with Vycryl Mesh as an Oncoplastic Repair of the Breast 
Lim YA, Lee YO, Kim HY, Kim HS, Kang HJ, Kim LS. Hallym Sacred Heart Hospital, College of Medicine, Hallym University, Anyang, Gyeonggi-do, Korea.

P2-15-12 Management Strategy of Early-Stage Breast Cancer Patients: With or without Axillary Lymph Node Dissection 
Yamamoto D, Tanaka K, Tsubota Y, Yosida H, Kanematsu S. Kansai Medical University, Hirakata, Japan; Ribbon-Roze Tanaka Kanji Clinic, Osaka, Japan.

P2-15-13 Oncologic and Cosmetic Outcome in Breast Cancer Patients Who Underwent “Moving Window” Operation 
Ohno Y, Noguchi M, Nakano Y, Noguchi M, Kosaka T. Kanazawa Medical University, Japan.

P2-16-01 A Multi-Centre Prospective Cohort Study Evaluating Health Related Quality of Life after Types of Immediate Latissimus Dorsi (LD) Breast Reconstruction 

P2-16-02 How Well Do Medical Oncologists and Plastic Surgeons Communicate about Their Patients? 
Milucky J, Deal A, Wu R, McNally RS, Anders C, Lee C. University of North Carolina at Chapel Hill, Chapel Hill, NC.

P2-16-03 Outcomes of Nipple-Sparing Mastectomy (NSM) and Immediate Reconstruction 
Chidester JR, Olson JR, Poruk KE, Marenco JJ, Matsen CB, Neumayer L, Agarwal J, Loma Linda University Medical Center, Loma Linda, CA; University of Utah School of Medicine, Salt Lake City, UT.

P2-16-04 Abdominal Wall Stability and Flap Complications Are Unaltered Following Deep Inferior Epigastric Perforator (DIEP) flap Breast Reconstruction Despite Increasing Body Mass Index: Analysis of 418 Patients and 639 Flaps 
Ochoa O, Chrysopoulo M, Nastala C, Ledoux P, Pisano S. Plastics Reconstruction and Microsurgical Associates of South Texas, San Antonio, TX.

P2-16-05 Delay of Adjuvant Chemotherapy after Elective Mastectomy and Immediate Reconstruction in Breast Conservation Candidates: A Matched-Pair Analysis 
Batty PN, Riley EC, Pan J, Crew JB, Lee K, Jain D, Kruse BJ, Quillo AR, Rai S, Dragun AE. James Graham Brown Cancer Center, University of Louisville School of Medicine, Louisville, KY.

P2-16-06 A Systematic Review of Standardised Clinical Outcomes and Patient Reported Outcome Measures (PROMS) in Breast Reconstruction 
Winters ZE, Chaudhry A, Benson JR. University Hospitals Bristol NHS Foundation Trust, Bristol, South West, United Kingdom; Cambridge University Hospitals NHS Foundation Trust, Cambridge, East Anglia, United Kingdom.

P2-16-09 Nipple-Areolar Sparing Mastectomy: Utility of the Lateral Inframammary Incision in Immediate Implant-Based Reconstructions 

P2-16-10 Practice Variations in Post-Mastectomy Breast Reconstruction: What Are the Roles of Clinical Factors, Access Barriers, and Delayed Reconstruction? 
Cox D, Milucky J, Dominik R, Lee C. University of North Carolina at Chapel Hill, Chapel Hill, NC.

P2-16-11 Role of Proper Patient Counselling in Combination with Effect of Socioeconomic Deprivation on the Rate of Immediate Breast Reconstruction after Mastectomy 
Chakrabarti M, Fitzgerald C, Obbond C, Weiler-Mithof E, Doughty J, Romics, JrL. Victoria Infirmary Glasgow; Glasgow Royal Infirmary; Western Infirmary Glasgow.

P2-16-12 Skin Reducing Nipple Sparing Mastectomy - A Treatment Option for Patients with Significant Ptosis or Macromastia 
Ott Young A, Alizadeh K, Hodyl C, Pronovost M, Davenport T, Yale New Haven Health System, Fairfield, CT; Long Island Plastic Surgical Group, Garden City, NY; South Nassau Communities Hospital, Oceanside, NY.

P2-16-13 Life after Mastectomy without Breast Reconstruction - Are the Needs of Caribbean Women Different? 

P2-16-14 Skin Sparing Mastectomy and Immediate Latissimus Dorsi Flap Reconstruction: Patient Reported Outcome and Factors Affecting the Highest Patient Satisfaction 

P2-16-15 Oncological Safety and Survival Rate According to Reconstruction Surgery in Advanced Breast Cancer after Neoadjuvant Chemotherapy 
Kim WW, Lee JJ, Nam KH, Jung JH, Chae YS, Yang JD, Lee YH, Park HY. Kyungpook National University, Hyoysung Hospital.

P2-16-16 Nipple Sparing Mastectomy and Immediate Breast Reconstruction: Critical Appraisal of Five Year, Single Centre Outcomes 
Westbroek D, Karat I, Min-Hui H, Gukas I, Daoud R, Laidlaw IJ. Frimley Park Hospital, Portsmouth Road, Frimley, Surrey, United Kingdom.

P2-16-07 A Randomised Controlled Trial To Evaluate the Role of Tisseel, a Fibrin Sealant on Seroma Formation in Latissimus dorsi Breast Reconstruction 

P2-16-08 Expander-Implant-Reconstruction - Aesthetic Outcome and Patients' Satisfaction in a 10-Year-Period 
Kern PA, Zarth F, Rainer K, Scherag AS, Mahdi R. Breast Center Dusseldorf Louis Hospital, Dusseldorf, North Rhine Westfalia, Germany; University Clinic of Essen, Essen, North Rhine Westfalia, Germany; University of Duisburg-Essen, Essen, Germany.

P2-16-07 A Randomised Controlled Trial To Evaluate the Role of Tisseel, a Fibrin Sealant on Seroma Formation in Latissimus dorsi Breast Reconstruction 

P2-16-08 Expander-Implant-Reconstruction - Aesthetic Outcome and Patients' Satisfaction in a 10-Year-Period 
Kern PA, Zarth F, Rainer K, Scherag AS, Mahdi R. Breast Center Dusseldorf Louis Hospital, Dusseldorf, North Rhine Westfalia, Germany; University Clinic of Essen, Essen, North Rhine Westfalia, Germany; University of Duisburg-Essen, Essen, Germany.

P2-16-09 Nipple-Areolar Sparing Mastectomy: Utility of the Lateral Inframammary Incision in Immediate Implant-Based Reconstructions 

P2-16-10 Practice Variations in Post-Mastectomy Breast Reconstruction: What Are the Roles of Clinical Factors, Access Barriers, and Delayed Reconstruction? 
Cox D, Milucky J, Dominik R, Lee C. University of North Carolina at Chapel Hill, Chapel Hill, NC.

P2-16-11 Role of Proper Patient Counselling in Combination with Effect of Socioeconomic Deprivation on the Rate of Immediate Breast Reconstruction after Mastectomy 
Chakrabarti M, Fitzgerald C, Obbond C, Weiler-Mithof E, Doughty J, Romics, JrL. Victoria Infirmary Glasgow; Glasgow Royal Infirmary; Western Infirmary Glasgow.

P2-16-12 Skin Reducing Nipple Sparing Mastectomy - A Treatment Option for Patients with Significant Ptosis or Macromastia 
Ott Young A, Alizadeh K, Hodyl C, Pronovost M, Davenport T, Yale New Haven Health System, Fairfield, CT; Long Island Plastic Surgical Group, Garden City, NY; South Nassau Communities Hospital, Oceanside, NY.

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Kim WW, Lee JJ, Nam KH, Jung JH, Chae YS, Yang JD, Lee YH, Park HY. Kyungpook National University, Hyoysung Hospital.

P2-16-16 Nipple Sparing Mastectomy and Immediate Breast Reconstruction: Critical Appraisal of Five Year, Single Centre Outcomes 
Westbroek D, Karat I, Min-Hui H, Gukas I, Daoud R, Laidlaw IJ. Frimley Park Hospital, Portsmouth Road, Frimley, Surrey, United Kingdom.

P2-17-01 Influence of Delayed Zoledronic Acid Initiation on Disease-Free Survival in Postmenopausal Women with Endocrine Receptor-Positive Early Breast Cancer Receiving Adjuvant Letrozole: Exploratory Analyses from the ZO-FAST Trial 
Coleman R, De Boer R, Eidtmann H, Neven P, von Minckwitz G, Martin N, Modi A, Bunded N. University of Sheffield, Sheffield, United Kingdom; Royal Melbourne Hospital, Victoria, Australia; University Frauenklinik, Kiel, Germany; UZ Gasthuisberg, Leuven, Belgium; German Breast Group, Frankfurt, Germany; Novartis Oncology, East Hanover, NJ; University Hospital of South Manchester NHS Foundation Trust, Manchester, United Kingdom.
**P2-17-02** Increased Progression Free and Overall Survival in Breast Cancer Patients with Menopausal Symptoms or Arthralgia/Myalgia during Adjuvant Treatment with Exemestane or Tamoxifen - Results of the German TEAM Trial
Hadji P, Kieback DG, Hasenburg A, Tams J, Ziller M, Philippus University, Marburg, Germany; Eiblandkliniken, Meissen, Germany; University Hospital Freiburg, Freiburg, Germany; ICRC-Weyer GmbH, Berlin, Germany.

**P2-17-03** Incomplete Uptake and Diminished Adherence to Endocrine Therapy in Women with Hormone Receptor Positive Breast Cancer 4 Years from Diagnosis: An Australian Cohort Study
Bell RJ, Fradkin P, Schwarz M, Davis SR. Monash University, Melbourne, VIC, Australia; Alfred Health, Melbourne, VIC, Australia.

**P2-17-04** Induction of Tamoxifen Metabolism by Rifampicin: A Worrying Drug-Drug Interaction
Binkhorst L, Loos WJ, de Jongh FE, Hamberg P, Goboai Moghadam-Helmantel IM, Jager A, Seynaeve C, Verweij J, van Gelder T, Mathijssen RH. Erasmus University Medical Center, Rotterdam, Netherlands; Erasmus University Medical Center - Daniel den Hoed Cancer Center, Rotterdam, Netherlands; Iziak Hospital, Rotterdam, Netherlands; Sint Franciscus Gasthuis, Rotterdam, Netherlands.

**P2-17-05** Antiprogestin Prolexel Suppresses Proliferation of Aromatase Overexpressing and Letrozole Resistant T47D Breast Cancer Cells

**P2-17-06** Patterns of Bone Density Evaluation in a Community Population Treated with Aromatase Inhibitors

**P2-17-07** Construction of a Predictive Model of Probability of Ovarian Function Recovery in a Series of Premenopausal Breast Cancer Patients with Chemotherapy-Induced Amenorrhea Switched to an Aromatase Inhibitor (AI) after Adjuvant Tamoxifen Perez-Fidalgo JA, Bermejo B, Pons V, Guzman C, Bosch A, Lluch A. Hospital Clinico Universitario, Valencia, Spain; Pfizer Spain, Alcobendas (Madrid), Spain.

**P2-17-08** Prospective Study of Aromatase Inhibitor Induced Bone Loss and Lipid Levels in Early Postmenopausal (PM) Hormone Receptor Positive (HR+) Breast Cancer (BC) Patients Treated with Adjuvant Letrozole Extended beyond 5 Years (yrs)

**P2-17-09** A Prospective Assessment of Loss of Grip Strength by Baseline BMI in Breast Cancer Patients Receiving Adjuvant Aromatase Inhibitors or Tamoxifen

**P2-18-01** The Magnitude of Trastuzumab Benefit in HER2-Positive (HER2+) Lobular Breast Carcinoma (BC): Results of a HERA Trial Sub-Group Analysis
Metzger O, Ploeger M, de Azambuja E, Viale G, Leyland-Jones B, Dowsett M, Gelber R, Gregoir E, Lui S, Sotiroiu C, Piccart M. Institut Jules Bordet, Université Libre de Bruxelles, Brussels, Belgium; Frontier Science (Scotland) Ltd, Kincraig, Kingsussie, United Kingdom; European Institute of Oncology, Milan, Italy; Emory University, Atlanta, Royal Marsden Hospital and Institute of Cancer Research, London, United Kingdom; Dana-Farber Cancer Institute, Boston; Roche, Basel, Switzerland.

**P2-18-02** Cardiovascular Outcomes of Patients on Adjuvant Weekly Paclitaxel (T) and Trastuzumab (H) for Node Negative, HER2 Positive Breast Cancer (BCA)
Dang C, Tolaney S, Najita J, Gelman R, Yardley D, Marcom K, Albain K, Rugo H, Miller K, Ellis M, Shapira I, Wolff A, Carely L, Vahdat L, Burdette-Radoux S, Budd T, Krop I, Burstein H, Hudis C, Winer E. Memorial Sloan-Kettering Cancer Center; Dana-Farber Cancer Institute; Tennessee Oncology and Sarah Cannon Research Institute; Duke University; Loyola University Medical Center; University of California-San Francisco Comprehensive Cancer Center; Indiana University; Washington University; North Shore-Long Island Jewish Medical Center; Johns Hopkins University; University of North Carolina; Weill Cornell Medical College; University of Vermont; Cleveland Clinic Taussig Cancer Institute Case.

**P2-18-03** Systemic Adjuvant Treatment of T1a and T1b N0M0 HER2+ Breast Carcinomas; an AERIO/UNICANSEY Study

**P2-18-04** Pilot Evaluation of Bevacizumab (Bev) in Combination with Docetaxel (T) and Cyclophosphamide (C) as Adjuvant Treatment (AdjRx) for Patients (pts) with Early Stage (ES) Breast Cancer (BrCa)

**P2-18-05** Cardiotoxicity Risks of Adjuvant Trastuzumab in Asian Breast Cancer Patients
Shih V, Chan A, Sim MH, Teo C, Chen W, Wong ZW. National Cancer Centre, Singapore; National University of Singapore, Singapore.

**P2-18-06** Conventional Trastuzumab Is an Antagonist of Natural Killer Cells: Making the Case for Fucose-Depleted Trastuzumab
Listinskij JJ, Siegal GP, Listskiny CM. University of Alabama at Birmingham, Birmingham, AL; Case Western Reserve University, Cleveland, OH.

**P2-19-01** Impact of Zoledronic Acid on Fractures, Bone Mineral Density and Bone Remodeling in the AZURE Trial (BIG 01-04)
Coleman R, Woodward E, Turner L, Marshall H, Collinson M, Dodwell D, Davies C, Bell R, Cameron D, Brown J. University of Sheffield, Sheffield, United Kingdom; University of Leeds, Leeds, United Kingdom; Andrew Love Cancer Centre, Geelong, Victoria, Australia; Western General Hospital, Edinburgh, United Kingdom.

**P2-19-02** Multidisciplinary Treatment of Pregnancy-Associated Breast Cancer
Meisel JL, Economy KE, Zabicki-Calvillo K, Gelber S, Kereakoglow S, Winer EP, Partridge AH, Mayer EL, Brigham and Women’s Hospital, Boston, MA; Dana-Farber Cancer Institute, Boston, MA.
**PD03-02** Prognostic and Predictive Predictors for Triple Negative Breast Cancer
Karn T, Pusztai L, Ruckhaberle E, Liedtke C, Schmidt M, Müller V, Galá T, Hänker L, Ahr A, Holtrich U, Ahr A, Kaufmann M. Goethe University, Frankfurt, Germany; University of Texas MD Anderson Cancer Center, Houston, TX; Institut Paoli-Calmettes (IPC), Marseille, France; World IBC Consortium; Contributed Equally.

**PD03-03** Identification of Transcription Factors Critical for the Growth of Basal Breast Cancer
Shepherd JH, Mazumdar A, Tsimelzon A, Hilsenbeck SG. Brown Phil. Baylor College of Medicine, Houston, TX; The University of Texas MD Anderson Cancer Center, Houston, TX.

**PD03-04** SuperPathway Analyses of Luminal and Basaloid Breast Cancers from the Cancer Genome Atlas (TCGA) Program
Yau C, Benz S, Sanborn JZ, Stuart J, Hausler D, Benz C. Buck Institute for Research on Aging, Novato, CA; UC Santa Cruz, Santa Cruz, CA.

**PD03-05** Withdrawn

**PD03-06** Basal-Like Breast Tumors Are Associated with Frequent Micro Genomic Aberrations in Important Cancer Genes
Chao H-H, He X, Perez CM. University of North Carolina - Chapel Hill, Chapel Hill, NC.

**PD03-07** Breast Cancer Heterogeneity and Treatment Resistance: Clues from Metaplastic Tumors
Felding-Habermann B, O’Sullivan DM, Lorger M, MacDermid D, Fernandez-Santidrian A, Steele JB, Telli ML, Jeffrey SS, Murray S, Torkamani A, Cunillie H, Vaughn S. The Scripps Research Institute, La Jolla, CA; Scripps Clinic, La Jolla, CA; Stanford University, Stanford, CA; Translational Genomics Research Institute, Phoenix, AZ.

**PD03-08** BRCA1-Like Triple Negative Tumors: Clinicopathological Variables and Chemosensitivity to Alkylating Agents
Wesseling J, Lips EH, Donk AMM, Smits RM, van Rijn CCM, Mulder L, Laddach N, Savola SA, Wessels LFA, Nederlof PM, Rodenhuis S, Imholz ALT. Netherlands Cancer Institute-Antoni van Leeuwenhoek Hospital, Amsterdam, Netherlands; Deventer Hospital, Deventer, Netherlands; MRC-Holland, Amsterdam, Netherlands.

**PD03-09** Breast Cancer Recurrence Risk Probed by Whole Transcriptome Next Generation Sequencing in 136 Patients

**PD03-10** Gene Modules and Response to Neoadjuvant Chemotherapy in Breast Cancer: A Meta-Analysis

**PD04-01** Predictors of Recovery of Ovarian Function during Aromatase Inhibitor (AI) Therapy
Henry NL, Banerjee M, Hayden J, Yakim E, Schott AF, Steams V, Partridge AH, Hayes DF. University of Michigan Medical School, Ann Arbor, MI; Johns Hopkins School of Medicine; Dana Farber Cancer Institute.

**PD04-02** Recovery of Ovarian Function in Breast Cancer Patients with Chemotherapy-Induced Amenorrhea Receiving Anastrozole in the Dutch DATA Study
Tjan-Heijnen VC, Smorenburg CH, de Graaf H, Erdkamp F, Honkoop A, Wals J, van Gastel S, van der Sangen M, Seynaeve C, Nortier JW, Borm G. Maastricht University Medical Centre, Netherlands; Medical Centre Alkmaar, Netherlands; Medical Centre Leeuwarden, Netherlands; Orbis Medical Centre, Netherlands; Isala Clinics, Netherlands; Artrium Medical Centre, Netherlands; Comprehensive Cancer Centre Netherlands Nijmegen, Netherlands; Catharina-Hospital, Netherlands; Erasmus University Medical Centre, Netherlands; Leiden University Medical Centre, Netherlands; Redbud University Nijmegen Medical Centre, Netherlands.
PD04-05  Body Image Issues in Young Breast Cancer Patients: The Impact of Chemotherapy, Hormone Treatment, and Surgery
Rosenberg SM, Tamimi RM, Gelber S, Kenakaloglu S, Borgan V, Currie S, Schapira L, Winer E, Partridge A. Harvard School of Public Health, Boston, MA; Dana-Farber Cancer Institute, Boston, MA; University of Colorado Cancer Center, Denver, CO; Beth Israel Deaconess Medical Center, Boston, MA; Massachusetts General Hospital, Boston, MA.

PD04-06  A Randomised Controlled Trial of Quality of Life and Fatigue after Support Group Intervention in Primary Breast Cancer Patients
Granstam Bjomeklett H, Lindemalm C, Ojutkangas M-L, Berglund A, Letocha H, Strang P, Bergkvist L. Centre for Clinical Research, Västerås, Sweden; Cancer Centre Karolinska, Solna, Sweden; Centre for Clinical Research, Central Hospital, Västerås, Sweden; Karolinska Institute, STH, Stockholm, Sweden; Karolinska Institute, Stockholm, Sweden.

PD04-07  Cognitive Function and Reproductive Hormones in Women Receiving Anastrozole
Bender CM, Sereika SM, Ryan CM, Berga SL. University of Pittsburgh, Pittsburgh, PA; Emory University, Atlanta, GA.

PD04-08  Altered Neurocognitive Responses Prior to Adjuvant Therapy for Breast Cancer: A Functional MRI Analysis of the Impact of Worry and Fatigue
Cimprich B, Hayes DF, Askren MK, Jung MS, Berman MG, Therrien B, Reuter-Lorenz PA, Zhang M, Peltier S, Noll DC. University of Michigan, Ann Arbor, MI.

PD04-09  Self-Reported Cognitive Attributes and Fatigue Improve over Long-Term Follow-Up in Breast Cancer Survivors; Some Cognitive Attributes Are Worse in Breast Cancer Survivors Than in Non-Cancer Controls
Hsu T, Ennis M, Hood N, Goodwin PJ. University of Toronto, Toronto, ON, Canada; Applied Statistician, Markham, ON, Canada; Mount Sinai Hospital, Toronto, ON, Canada; Mount Sinai and Princess Margaret Hospitals, Samuel Lunenfeld Research Institute, University of Toronto, Toronto, ON, Canada.

9:00 am–9:30 am  PLENARY LECTURE 2
Exhibit Hall D

Macrophages as Novel Targets for Therapy in Breast Cancer
Lisa M. Coussens, PhD
University of California San Francisco
San Francisco, CA

9:30 am–11:15 am  GENERAL SESSION 3
Exhibit Hall D

9:30  S3-1. Update of International Breast Cancer Study Group trial 23-01 to compare axillary dissection versus no axillary dissection in patients with clinically node negative breast cancer and micrometastases in the sentinel node

9:45  S3-2. Neoadjuvant chemotherapy adapted by interim response improves overall survival of primary breast cancer patients – results of the GeparTrio Trial

10:00  S3-3. Association of PTEN loss and PIK3CA mutations on outcome in HER2- metastatic breast cancer patients treated with first-line lapatinib plus paclitaxel or paclitaxel alone
Xu B-H, Guan Z-Z, Shen Z-Z, Tong Z-S, Yang J-Y, DeSilvio M, Leith G, Ellis C. Cancer Hospital, CAMS & PUMC, Sun Yat-Sen Medical University Cancer Center, Shanghai Cancer Center, Fudan University; Tianjin Medical University Cancer Institute and Hospital; Hospital Affiliated to Military Medical Science; Beijing 301 PLA Hospital; GlaxoSmithKline.

10:15  S3-4. ER downregulation with fulvestrant in combination with pan-PI3K inhibitor BKM120 synergizes against ER+/PI3K-mutant breast cancer xenografts in vivo
Miller TW, Fox EM, Balke JM, Meszoly IM, Sanders ME, Kuba MG, Wagle N, Garraway LA, Maira S-M, Arteaga CL. Vanderbilt University, Nashville, TN; Dana-Farber Cancer Institute, Boston, MA; Novartis Inst. for Biomedical Research, Basel, Switzerland.

10:30  S3-5. Next generation sequencing reveals co-activating events in the MAPK and p13k/akt pathways in metastatic triple negative breast cancers

10:45  S3-6. Neoadjuvant chemotherapy of paclitaxel with or without Rad001: Results of the non-responder part of the GEPRIQUINTO study (GBG 44)

11:00  S3-7. Everolimus for postmenopausal women with advanced breast cancer: updated results of the BOLERO-2 phase III trial
Hortobagyi GN, Piccart M, Rugo H, Harris C, Campone M, Noguchi S, Grant M, Pritchard KI, Vittori L, Mukhopadhyay P, Sahnoud T, Lebwohl D, Baselga J. The University of Texas, MD Anderson Cancer Center, Houston, TX; Jules Bordet Institute (Institut Jules Bordet), Brussels, Belgium; University of California, San Francisco Helen Diller Family Comprehensive Cancer Center, UCSF, San Francisco, CA; Sarah Cannon Research Institute, Nashville, TN; Institut de Cancérologie de l’Ouest - René Gauduchaud, Centre de Recherche en Cancérologie, Nantes Saint Herblain, France; Osaka University, Department of Breast and Endocrine Surgery, Osaka, Japan; Comprehensive Cancer Center, Medical University of Vienna, Vienna, Austria; Sunnybrook Odette Cancer Centre and the University of Toronto, Toronto, Canada; Novartis Pharma AG, Basel, Switzerland; Novartis Pharmaceuticals Corporation, East Hanover, NJ, Massachusetts General Hospital Cancer Center and Harvard Medical School, Boston, MA.
11:15 am–12:00 pm
WILLIAM L. MCGUIRE MEMORIAL LECTURE
Exhibit Hall D
Sponsored by Cancer Therapy & Research Center
Translating genomic insights into improved breast cancer management
Joe Gray, PhD
Oregon Health & Science University
Portland, OR

12:00 pm–1:35 pm
LUNCH

12:15 pm–1:15 pm
PRODUCT THEATRE
Exhibit Hall C – Exhibit Area
Abraxane
Sponsored by Celgene Corporation

12:30 pm–1:35 pm
CASE DISCUSSION 1
Ballroom A
Moderator: Mothaffar Rimawi, MD
Baylor College of Medicine
Houston, TX
Panelists:
John Benson, MD
Cambridge University
Cambridge, UNITED KINGDOM
Lisa Carey, MD
University of North Carolina
Chapel Hill, NC
Daniel Hayes, MD
University of Michigan
Ann Arbor, MI
Wendy Woodward, MD
MD Anderson Cancer Center
Houston, TX
Kay Wissmann
IL Deadline Action Network (NBCC)
Carbondale, IL

12:30 pm–1:35 pm
BASIC SCIENCE FORUM
Ballroom B
Estrogen Receptor
Moderator: Suzanne A.W. Fuqua, PhD
Baylor College of Medicine
Houston, TX
Steroid receptor cistromics
Myles Brown, MD
Dana-Farber Cancer Institute
Boston, MA
Nuclear receptor coactivators: Physiology and disease
Bert W. O’Valley, MD
Baylor College of Medicine
Houston, TX

1:45 pm–3:15 pm
MINI-SYMPOSIUM 1
Ballroom B
Tumor Heterogeneity and Metastasis
Moderator: Rachel Schiff, PhD
Baylor College of Medicine
Houston, TX
Imaging cancer invasion and experimental therapy response:
How to overcome resistance niches
Peter Friedl, MD, PhD
Radboud University Nijmegen
HB Nijmegen, NETHERLANDS
Tumor-engrained neutrophils inhibit breast cancer metastasis
seeding in the lung
Robert Beneza, PhD
Memorial Sloan-Kettering Cancer Center
New York, NY
Tumor heterogeneity and metastasis
Matthew J. Ellis, MB, BChir, PhD
Washington University School of Medicine
St. Louis, MO

1:45 pm–3:15 pm
MINI-SYMPOSIUM 2
Exhibit Hall D
Environmental Exposures, Epigenetics and Epidemiology
Moderator: Cheryl Lyn Walker, PhD
UT MD Anderson Cancer Center
Houston, TX
Epidemiology of preeclampsia and other early exposures on breast cancer risk
Michele R. Forman, PhD
UT Austin
Austin, TX
Environ exposures, epigenetics & epidemiology influence of environmental factors on pubertal maturation and breast cancer etiology
Robert A. Hiatt, MD, PhD
University of California, San Francisco
San Francisco, CA
Breast cancer and the environment: Developmental reprogramming of cancer susceptibility by early life environmental exposures
Cheryl Lyn Walker, PhD
UT MD Anderson Cancer Center
Houston, TX

3:15 pm–5:15 pm
GENERAL SESSION 4
Exhibit Hall D
Overdiagnosis in breast cancer screening: Methodological considerations of current estimates
Smith RA, Duffy SW. American Cancer Society, Atlanta, GA; Queen Mary University of London, London, United Kingdom.
Risk of contralateral breast cancer in BRCA1/2 carriers compared to non-BRCA1/2 carriers in an unselected cohort
van den Broek AJ, Schmidt MK, Tollenaar RAEM, van ’t Veer LJ, van Leeuwen FE. Netherlands Cancer Institute, Amsterdam, Netherlands; Leiden University Medical Center, Leiden, Netherlands.
3:45  S4-3. Prospective comparison of risk assessment tools in early breast cancer (recurrence score, uPA/PAI-1, central grade, and luminal subtypes): Final correlation analysis from the phase III WSG-plan B trial
Gluz O, Kreipe H, Degenhardt T, Kates R, Christgen M, Liedtke C, Shakh S, Clemens M, Markmann S, Uefer C, Augustin D, Thomssen C, Nitz U, Harbeck N. West German Study Group, Moenchengladbach, Germany; Medizinische Hochschule, Hannover, Germany; University of Muenster, Muenster, Germany; Genomic Health Inc, Redwood City; Klinikum Mutterhaus, Trier, Germany; Klinikum Suedstadt, Rostock, Germany; Gynecology Practice, Hildesheim, Germany; Klinikum Deggendorf, Deggendorf, Germany; University of Halle/Saale, Halle/Saale, Germany; Bethesda Clinics, Moenchengladbach, Germany; University of Cologne, Cologne, Germany.

4:00  S4-4. Clarifying the risk of breast cancer in women with atypical breast lesions
Coopey SB, Mazzola E, Buckley JM, Sharko J, Belli AK, Kim EMH, Polufriasgoff F, Parmigiani G, Garber JE, Smith BL, Gadd MA, Specht MC, Guidi AJ, Roche CA, Hughes KS. Massachusetts General Hospital, Boston, MA; Dana-Farber Cancer Institute, Boston, MA; Newton-Wellesley Hospital, Newton, MA; Wayne State University, Detroit, MI.

4:15  S4-5. Comparison of PAM50 risk of recurrence (ROR) score with oncostyoxDx and IH4C for predicting residual risk of DFS and distant DFS after endocrine therapy: A TransATAC study

4:30  S4-6. A quantitative multigene RT-PCR assay for predicting recurrence risk after surgical excision alone without irradiation for ductal carcinoma in situ (DCIS): A prospective validation study of the DCIS score from ECOG ES194
Solin LJ, Gray R, Baehner FL, Butler S, Badve S, Yoshizawa C, Shakh S, Hughes L, Sledge G, Davidson N, Perez EA, Ingle J, Sparano JA, Wood W. Albert Einstein Medical Center, Philadelphia, PA; Eastern Cooperative Oncology Group, Boston, MA; Genomic Health, Inc., Redwood City, CA; Indiana University, Indianapolis, IN; The Hope Center, Cartersville, GA; University of Pittsburgh, Pittsburgh, PA; Mayo Clinic Jacksonville, Jacksonville, FL; Mayo Clinic Rochester, Rochester, MN; Albert Einstein College of Medicine Bronx, NY; Emory University, Atlanta, GA.

4:45  S4-7. Results of a randomized, double-blind, multicenter, placebo-controlled study of adjuvant lapatinib in women with early-stage ErbB2-overexpressing breast cancer
Goss P, Smith I, O’Shaughnessy J, Ejlertson B, Kaufmann M, Boyle F, Buzdar A, Fumoleau P, Gradishar W, Martin M, Moyer B, Piccart-Gebhart M, Pritchard K, Aktan G, Rappold E, Williams L, Finkelstein D, Massachusetts Gen Hosp, Boston MA; Royal Marsden Hosp, London, UK; Baylor Sammons Cancer Ctr, Dallas TX; Rigshospitalet, Copenhagen, Denmark; JW Goethe-Universitat, Frankfurt, Germany; Royal North Shore Hosp, Sydney, Australia; UT MD Anderson Cancer Ctr, Houston TX; Centre GF Leclerc, Dijon, France; Northwestern Univ, Chicago IL; Hosp Univeristario San Carlos, Madrid, Spain; Jules Bordet Inst, Brussels, Belgium; Toronto-Sunnybrook Regional Cancer Ctr, Toronto, Ontario; GlaxoSmithKline, Collegeville PA and Uxbridge, UK.

5:00  S4-8. First results of AVEREL, a randomized phase III trial to evaluate bevacizumab (BEV) in combination with trastuzumab (H) + docetaxel (DOC) as first-line therapy for HER2-positive locally recurrent/metastatic breast cancer (LR/mBC)

5:15 pm–7:00 pm  POSTER SESSION 3 & RECEPTION
Exhibit Halls A-B

Tumor Cell Biology: Novel/Emerging Therapeutic Targets
P3-01-01  Geminin Overexpression Prevents the Completion of Topoisomerase Ila Chromosome Decatenation Leading to Aneuploidy in Human Mammary Epithelial Cells
Eshamy HM, Gardner L, Malik R, Shinmu Y, Mullins N. University of Mississippi Medical Center, Jackson, MS.

P3-01-02  EC 304: A Novel Progesterone Receptor (PR) Antagonist Inhibits Transcription and Induces Cell Cycle Arrest in T47D Breast Cancer Cells
Blakkaran JS, Kesavaram N, Nicksk JJ, VandeBerg JL, Nair HB. Texas Biomedical Research Institute, San Antonio, TX, Evestra, Inc., San Antonio, TX.

P3-01-03  The Hominoid-Specific Gene SHON Is Oncogenic in Human Mammary Carcinoma

P3-01-04  Differential Impact of Gefitinib and PLX4720 on Proliferation of MCF10A and Isogenic Lines as Measured with a Metastasis Expression Score

P3-01-05  Wnt Signaling: A New Target for Treatment and Prevention of Endocrine Resistant Breast Cancer?
Micaleff RA, Smith C, Barrow D, Nicholson RI, Gee JMW, Barratt-Lee PJ, Hiscox SE. Cardiff University, Cardiff, Wales, United Kingdom; Velindre NHS Trust, Cardiff, Wales, United Kingdom.

P3-01-06  NF-kB Inhibition Promotes Radiosensitivity of Breast Cancer Cells in Three-Dimensional Culture through Abating β1-Integrin Expression
Ahmed KM, Zhang H, Park CC. Ernest Orlando Lawrence Berkeley National Laboratory, Berkeley, CA.

P3-01-07  A Murine Xenograft Model for the Study of Ciclooxygenase-2 Expression and Function in Human Breast Cancer
Solano AR, Orlando UD, Garona J, Ripoli GV, Duarte AB, Maloberti PM, Avagnina MA, Alonso DF, Gomez DE, Pedesta EJ. School of Medicine University of Buenos Aires, Buenos Aires, Argentina; University of Quilmes, Quilmes, Buenos Aires, Argentina; Center of Medical Education and Clinical Investigation Norteño Quirino, Buenos Aires, Argentina.
P3-01-08  In Vitro and In Vivo Antitumor Activity of the Aurora and Angiogenic Kinase Inhibitor ENMD-2076 in Triple-Negative Breast Cancer Models
Diamond JR, Erkkhardt SG, Tan AC, Selby HM, Newton TP, Pitts TM, Bray MR, Fletcher GC, Tentler JJ. University of Colorado Cancer Center, Aurora, CO; EntreMed, Inc, Toronto, ON, Canada.

P3-01-09  Oncogenic Activation of HSF1 Enables the Malignant Progression of Breast Carcinoma
Mendillo ML, Santagata S, Koeva M, Fraenkel E, Ince TA, Whitessell L, Lindquist S. Whitehead Institute for Biomedical Research, Cambridge, MA; Brigham and Women’s Hospital, Boston, Boston, MA; Massachusetts Institute of Technology, Cambridge, MA; University of Miami Miller School of Medicine, Miami, FL; Howard Hughes Medical Institute, Cambridge, MA.

P3-01-10  DMBA-Breast Cancer in Diet Induced Obesity (DIO) and Lean Mice Is Related to Leptin Signaling
Gillespie C, Penichet MG, Colbert LS, McGlothen T, Guo S, Zhou W, Gonzalez-Perez RR. Morehouse School of Medicine, Atlanta, GA; Shenyang Medical College, Liaoning Pro, P.R., China.

P3-01-11  Increased Gene Copy Number of c-KIT and VEGFR2 at 4q12 in Primary Breast Cancer Is Related to an Aggressive Phenotype and Impaired Prognosis

P3-01-12  Prognostic Impact of RANK, RANKL and OPG Gene Expression in ER Positive Primary Breast Cancer
Ruckhaeberle E, Mueller V, Schmidt M, Saengar N, Hanker L, Gaetje R, Ahr H, Holtrich U, Karm T, Rooy A, Kaufmann M. Department of Gynecological Oncology, Frankfurt, Germany; Department of Obstetrics and Gynecology, Hamburg, Germany; Department of Obstetrics and Gynecology, Mainz, Germany; Department of Obstetrics and Gynecology, Hornburg/Saar, Germany.

P3-01-13  Leptin Modulates the Response to Tamoxifen Treatment in ER-Positive Breast Cancer Cell Lines
Yoon CK, Han W, Kim S-W, Kim DW, Kang E, Noh D-Y. Seoul National University Bundang Hospital, Seoul National University College of Medicine, Seongnam, Korea; Seoul National University College of Medicine, Seoul, Korea.

P3-01-14  RANK and RANK Ligand (RANKL) Expression in Invasive Breast Carcinoma and Human Breast Cancer Cell Lines

P3-01-15  The Role of Src Homology Phosphotyrosyl Phosphatase-2 in Basal-Type/Triple-Negative Breast Cancer - Implications for Targeted Therapy
Agase YM, Matafka F, Hartman ZR. West Virginia University School of Medicine, Morgantown, WV.

P3-01-16  Expression of Basal Markers in Correlation with PTEN and BRCA 1 in Triple Negative Breast Carcinomas (TN) Treated with Chemotherapy Versus PARP Inhibitors
Chisvukula M, Carter G, Puhalisa S, Magee Women’s Hospital of Pittsburgh, Pittsburgh, PA; Magee Women’s Hospital of UPMC, Pittsburgh, PA.

P3-01-17  Cadherin-11 as a therapeutic target in poor prognosis breast cancers
Assensia S, Gudny Audvil JM, Dakshnamurthy S, Hampel C, Brown M, Anastadiadis P, Brenner M, Byers SW. Lombardi Comprehensive Cancer Research Center, Georgetown University Medical Center, Washington, DC; Mayo Clinic, Jacksonville, FL; Brigham and Women’s Hospital, Harvard University, Boston, MA.

P3-01-18  Amplification of Anaplastic Lymphoma Kinase (ALK) as a common genetic alteration in Inflammatory Breast Cancer
Robertsion FM, Petricoin EF, Chu X, Mu Z, Jin J, Circo R, Fernandez SV, Albaum K, Zook M, Sun G, Wulkkhuji J, Liotta LA, Ye Z, Krishnamurthy S, Luo AZ, Lui H, Wright MC, Woodward WA, Banks SH, Cristofanilli M. The University of Texas MD Anderson Cancer Center, Houston, TX; George Mason University, Manassas, VA; Fox Chase Cancer Center, Philadelphia, PA; The University of Nevada School of Medicine and Nevada Cancer Institute, Reno and Las Vegas, NV.

P3-01-19  PEGPH20 depletion of pericellular hyaluronan sensitizes high hyaluronan-producing tumor cells in antibody-dependent cell-mediated cytotoxicity

Tumor Cell Biology: New Agents and Mechanisms

P3-02-01  A Novel Bispecific, Hexavalent, Antibody (HexAb) Inhibits Anchorage-Independent Growth and Reduces Invasiveness of Triple-Negative Breast Cancer Cell Lines In Vitro

P3-02-02  Novel Ranpirnase-Based ImmunorNases Display Potent Cytotoxicity in Diverse Human Breast Cancer Cell Lines

Tumor Cell Biology: MicroRNAs

P3-03-01  Low Expression of microRNA-210 Is an Independent Good Prognostic Factor in Japanese Triple-Negative Breast Cancer Patients
Toyama T, Kondo N, Endo Y, Sugihara H, Yoshimoto N, Iwasa M, Takahashi S, Iwase H, Fuji Y, Yamashita H. Nagoya City University Graduate School of Medical Sciences, Nagoya, Aichi, Japan; Kumamoto University Graduate School of Medical Sciences, Kumamoto, Japan.

P3-03-02  Higher Expression Levels of Circulating miR-21, miR-19a and miR-10b Are Associated with High Risk Features in Breast Cancer
Anfossi S, Giordano A, Cohen EN, Gao H, Cristofanilli M, Valero V, Alvarez RH, Hortobagyi GN, Woodward W, Ueno NT, Lee B-N, Reuben JM. The University of Texas MD Anderson Cancer Center, Houston, TX; Fox Chase Cancer Center, Philadelphia, PA.

P3-03-03  Congruence between Patterns of microRNA Expression and Histologic Grading of Invasive Breast Carcinomas
Ellsworth DL, Croft DT, Field LA, Deyarman B, Kane J, Ellsworth RE, Hooke JA, Shriver CD. Windber Research Institute, Windber, PA; George Mason University, Manassas, VA; Fox Chase Cancer Center, Philadelphia, PA.

P3-03-04  Targeted Modulation of HER Receptor Signaling in Breast Cancer Using miR-21

P3-03-05  Identification of miRNAs and Their Associated Target Genes Involved in Endocrine Resistance in Breast Cancer
Healy NA, Creighton CJ, Fu X, Tismatch A, Hilsenbeck SG, Miller N, Kerin MJ, Osborne CK, Schiff R. Baylor College of Medicine, Houston, TX; National University of Ireland, Galway, Ireland.
P3-03-06  Regulation of microRNA Expression by Autocrine Human Growth Hormone in Breast Cancer Cells
Pertzinger M, Shasti BG, Liu D-X, Zhu T, Lobe PE, Perry JK. University of Auckland, Auckland, New Zealand; University of Science and Technology of China, Hefei, Anhui, China; National University of Singapore, Singapore.

P3-03-07  A Novel Approach Integrating microRNA and mRNA Signatures of HMAPK Signaling Is Highly Predictive of ER-Status and Outcome in Breast Cancer - Role of HMAPK microRNAs in Repression of ER and p27
Miller P, Clarke J, Koru-Sengul T, Brinkman J, El-Ashy D. University of Miami, Miami, FL.

P3-03-08  microRNAs in Mammary Stem-Like Cells and Triple-Negative Breast Cancer; Conserved Functions and Treatment Potential
Williams C, Aydogdu E, Katchy A, Lin C-Y, Haldosen L-A, Helguro L. University of Houston, Houston, TX; Karolinska Institute, Stockholm, Sweden; University of Aveiro, Aveiro, Portugal.

P3-03-09  miR-944 is down-regulated in HER2 positive breast cancer tumors: identification of putative mRNA targets by transcriptome analysis
Forseca MA, Romero SL, Hidalgo A, Rodriguez S, Bautista V, Marchat LA, Perez-Plascencia C, Arechaga E, Lopez-Camarillo C. Universidad Autonoma de la Ciudad de Mexico, Mexico, DF, Mexico; Instituto Nacional de Medicina Genomica, Mexico, DF, Mexico; Instituto de Enfermedades de la mama FUCAM, Mexico, DF, Mexico; Escuela Nacional de Medicina y Homeopatia del IFN, Mexico, DF, Mexico; Instituto Nacional de Cancerologia, Mexico, DF, Mexico.

Tumor Cell Biology: Molecular Profiles

P3-04-01  Molecular Characterization of African Breast Cancer; Results from a Large Tissue Microarray Study
Shaaban A, Hatfield A, Omoniyi Esan GO, Komolafe AO, Daramola A, Pathak D, D’Cruz N, Alizadeh Y, Lewis P, Titloie NA. St James’s Institute of Oncology, Leeds, United Kingdom; Obafemi Awolowo Teaching Hospital Complex, Ille-Ife, Nigeria; Swansea School of Medicine, Swansea, United Kingdom; College of Medicine University of Lagos/University Teaching Hospital, Lagos, Nigeria.

P3-04-02  Bevacizumab Treatment Alters Intrinsic Subtypes in a VEGF-Reinforced Xenograf Model of ER-Positive Breast Cancer
Gokmen-Polar Y, Toroni RA, Goswami C, Sanders KL, Sirimalle U, Mehta R, Li L, Ivan M, Badve S, Sledge GW. Indiana University School of Medicine, Indianapolis, IN.

P3-04-03  Identification of Hormone-Responsive Genes as Biomarkers for Menstrual Cycle Phases and Menopausal Status

P3-04-04  Patterns of Distant Metastasis According to the Molecular Subtypes of Breast Cancer; Results of 529 Breast Cancer Patients
Hwang MJ, Seo YJ, Lee JH, Son GT, Choi JE, Bae YK, Kang SH, Lee SJ. Yeungnam University College of Medicine, Daegu, Republic of Korea.

P3-04-05  Expression Profiles of RANK and RANKL mRNA and Protein in the Mammary Gland of Female Cynomolagus Monkeys after Long-Term Treatment with Menopausal Hormone Therapy
Branstretter D, Wood CE, Rohrbach K, Borgenik H, Dougall WC. Amgen Inc., Seattle, CA; Wake Forest School of Medicine, Winston-Salem, NC.

P3-04-06  Comparison of MammaPrint, BluePrint, and TargetPrint with Clinical Outcomes in Patients with Breast Cancer: Findings from a Prospective United States Cohort
Nguyen B, Sinha R, Kerlin D, Barone J, Garcia A, Yao K, Rivera E, Stork-Sloots L, Deck K. Long Beach Memorial Health Care, Long Beach, CA; Rockwood Clinic, Spokane, WA; John Muir, Walnut Creek, CA; Comprehensive Breast Care of San Diego and Sharp Memorial Hospital, San Diego, CA; University of Southern California, Los Angeles, CA; North Shore University Health System, Chicago, IL; The Methodist Hospital/Weill Cornell University, Houston, TX; Agenda Inc, Irvine, CA; Saddleback Memorial Medical Center, Laguna Hills, CA.

P3-04-07  Physiological Concentrations of Genistein and 17β-Estradiol Inhibit MDA-MB-231 Breast Cancer Cell Proliferation by Increasing Bax/Bcl2 Ratio and Decreasing pERK1/2 Expression
Rajah TT, Peine KJ, Xu N, Serret CA. DePaul University, Chicago, IL.

P3-04-08  Increased cellular expression activity of minimal residual disease in breast cancer after surgery
Pachmann K, Carl S, Camara O. Friedrich Schiller University Jena, Jena, Germany.

Tumor Cell Biology: Tumor Heterogeneity/Molecular Subclassification

P3-05-01  Gene Profiling of Histopathologically Characterized Apocrine Breast Cancers

P3-05-02  Subtype-Specific Co-Occurrence of Atypical Hyperplasia and In Situ Carcinoma with Invasive Breast Cancers
Kovatch AJ, Kwecher L, Chen Y, Bekhash A, Hooke JA, Shriver CD, Mural RJ, Hu H. Windber Research Institute, Windber, PA; Walter Reed Army Medical Center, Washington, DC; MDR Global Systems LLC, Windber, PA.

P3-05-03  Transcriptomic Validation of Molecular Classification of Invasive Ductal Carcinoma Based on Immunohistochemical Markers and Grade

P3-05-04  Changes in Recurrence Risk of Breast Cancer Intrinsic Subtypes over Time

P3-05-05  Cyclin D1 Gene Amplification Is Rarely Heterogeneous in Breast Cancer

P3-05-06  Progression of Breast Cancer Molecular Subtypes through Different Clinical Stages

P3-05-07  Genetic Heterogeneity of Amplification Status in Breast Invasive Carcinoma with 2+ HER2 Immunostaining: What Can We Learn?
Valent A, Delaloge S, Fechoumi M, Bernheim A, Mathieu M-C. Institut Gustave Roussy, Villejuif, France, Metropolitan.
P3-05-08 Hormone Receptor Heterogeneity in Ductal Intraepithelial Neoplasia (Ductal Carcinoma In Situ) of the Breast
Sweedan M, Flynn G, Bossuyt V, Janzen D, Chagpar AB. Yale University School of Medicine, New Haven, CT.

P3-05-09 Comparison of Clinical Features and Patterns of Recurrence in Triple Negative Breast Cancers in Relation to Other Breast Cancers

P3-05-10 Standardized Quantitative Methods for Investigating the Intratumor Heterogeneity of HER2 in FFPE Breast Cancer Specimens Utilizing the Vectra System, inForm and AQUA Technology
Hoyt CC, Gustavson MD, Davis WL, Lane KA, Scott CG, Graves, Jr LL. Caliper Life Sciences, Hopkinton, MA, HistRx, Branford, CT.

Tumor Cell Biology: Genomics

P3-06-01 Next Generation RNA Sequencing Reveals Changes in Gene Expression and Alternative Splicing upon Brief Exposure to Therapy in Early Breast Cancer
Varadan V, Kamalakaran S, Jansenski A, Banerjee N, Leonz-Geyda K, Bossuyt V, Flowers D, Sikov W, Abu-Khalaf M, Rizack T, Dimitrova N, Harris LN. Philips Research North America, Briarcliff Manor, NY; Yale University School of Medicine, New Haven, CT, Brown University School of Medicine, Providence, RI.

P3-06-02 Identification of Redundant, Tumor Subtype Specific Fusion Transcripts in Primary Breast Tumors

P3-06-03 Hypodiploidy, 1pter Loss and Inactive X Chromosome Inactivation Are Associated with BRCA1 Somatic or Germline Determination and Other Basal-Like Breast Carcinomas: Proposal for a New BRCA1 Genomic Signature

P3-06-04 Sno/miRNA Expression Via Next Generation Sequencing: Variation in Patients before and after Treatment
Banerjee N, Kamalakaran S, Varadan V, Jansenski A, Leonz-Geyda K, Bossuyt V, Flowers D, Sikov W, Abu-Khalaf M, Rizack T, Harris LN. Philips Research North America, Briarcliff Manor, NY; Yale University School of Medicine, New Haven, CT, Brown University School of Medicine, Providence, RI.

P3-06-05 Comparison of Oncotype DX® Recurrence Scores between Surgical and Core Biopsy Specimens in Breast Cancer Patients
Stull TS, Goodwin MC, Anderson JM, Baehner FL, Sing AP, Yoshizawa CN, Barrio AV, Frazier TG. The Bryn Mawr Hospital, Bryn Mawr, PA; Genomic Health, Inc, Redwood City, CA.

P3-06-06 Comparison of Gene Expression Profiles of Lymph Node Positive and Lymph Node Negative ER Positive Breast Tumors in Pre- and Postmenopausal Women
Rapun PB, Brilliart G, Devarin B, Vranckx L, Hu H, Hooij JK, Shinver CD, Mural RJ. Windber Research Institute, Windber, PA; Walter Reed Medical Center, Washington, DC.

P3-06-07 Integrated Genomic and Pathway Analysis Reveals Key Pathways across Breast Subtypes
Benz S, Sanborn JZ, Vaske C. FireSc Genomics, LLC, Santa Cruz, CA.

P3-06-08 SCAN-B: An Accelerated Translational Pipeline from Profile to Prognosis, and Prediction for Individual Breast Cancer Patients

Detection and Diagnosis: Auxillary Staging and Sentinel Nodes

P3-07-01 Are the Findings of ASCO/Z0011 Applicable to District General Hospital Breast Unit - And How Should They Change Our Practice?

P3-07-02 Prediction of Non-Sentinel Lymph Node Status in Breast Cancer Patients with a Micrometastatic Sentinel Lymph Node Determined by the One Step Nucleic Acid Amplification (OSNA) Assay
Di Filippo C, Casini B, Gallo E, Terenato I, Botti C, Mottolese M, Pescarmona E, Marandino F, Buglioni S. Regina Elena National Cancer Institute, Rome, Italy; Regina Elena National Cancer Institute, Italy.

P3-07-03 One-Step Nucleic Acid Amplification (OSNA) for the Diagnosis of Sentinel Lymph Nodes of Breast Cancer - Results of the China Multicenter Study CBCSG-001c
Wang YS, Ouyang T, Wu J, Liu YH, Cao XC. Shandong Cancer Hospital & Institute, Jinan, Shandong, China; Beijing University Cancer Hospital, Beijing, China; Fudan University Cancer Hospital, Shanghai, China; Guangdong General Hospital, Guangzhou, Guangdong, China; Tianjin Medical University Cancer Hospital, Tianjin, China.

P3-07-04 Does Omission of Axillary Dissection after a Positive Sentinel Node Biopsy Influence Indication to Adjuvant Chemotherapy in Operable Breast Cancer Patients?

P3-07-05 Nomogram Incorporating SLN Metastasis Size Provides the Most Accurate Prediction of Non-SLN Involvement in Breast Cancer Patients

P3-07-06 Prognostic Utility of Upfront Nodal Staging Prior to Neoadjuvant Chemotherapy: The UAB Experience

P3-07-07 Effect of Sentinel Lymph Node Biopsy without Axillary Lymph Node Dissection on Overall Survival in Patients with T1 or T2 Node-Positive Breast Cancer: A Report from the Korean Breast Cancer Society
Park HS, Chae BJ, Song BJ, Jung SS, KBCS Investigators. The Catholic University School of Medicine, Seoul, Korea, Korean Breast Cancer Society.

P3-07-08 Accurate Staging of Axillary Lymph Nodes from Breast Cancer Patients Using a Novel Molecular Method

P3-07-09 Prediction of Additional Nodal Metastasis in Breast Cancer Patients with a Positive Sentinel Node Biopsy: A New Nomogram Including HER2 Status
P3-07-10 Sentinel Lymph Node Navigation Surgery Using Combination of Indocyanine Green Fluorescence and Blue Dye for Breast Cancer Patients
Takahashi M, Hayashida T, Jinno H, Kitagawa Y. Keio University School of Medicine, Tokyo, Japan.


P3-07-12 Value of Preoperative Ultrasound-Guided Axillary Lymph Node Biopsy for Preventing Futility Sentinel Node Procedures in Breast Cancer: A Systematic Review and Meta-Analysis
Diepstraten SC, Sever AR, Buckens CF, Veldhuis WB, van den Bosch MA, Mall WP, Verkooijen HM. University Medical Center Utrecht, Netherlands; Maidstone Hospital, United Kingdom.

P3-07-13 Validation and Comparison of Models To Predict Nonsentinel Lymph Node Metastasis in Chinese Breast Cancer Patients with Positive Sentinel Lymph Nodes
Chen K, Jia W, Zeng Y, Fan M, Su F, Li S. Sun Yat-sen Memorial Hospital, Sun Yat-sen University, Guangzhou, Guangdong, China.

P3-07-14 Sensory Disturbance of the Ipsilateral Upper Arm after Breast Cancer Surgery with Sentinel Node Biopsy Alone Compared with Axillary Dissection - A Prospective Study
Ohsumi S, Kiyoto S, Takahashi M, Hara F, Takabatake D, Takashima S, Aogi K, Shimozuma K. NHK Shikoku Cancer Center, Matsuyama, Ehime, Japan; Ritsumeikan University, Kusatsu, Shiga, Japan.

P3-07-15 Sequential Peri-Areolar and Peri-Tumoural SPECT/CT Lymphoscintigraphy Has Identified High Rates of Discordance in Both Axillary and Internal Mammary Sentinel Lymph Node Mapping

P3-07-16 The Efficacy of Axillary Node Preserving Surgery Using Axillary Reverse Mapping for Preventing Lymphedema in Patients with Breast Cancer
See YJ, Hwang MJ, Lee JH, Son GT, Choi JE, Bae YK, Kang SH, Lee S.J. Yeungnam University College of Medicine, Daegu, Republic of Korea.

P3-07-17 Optimal Number of Sentinel Lymph Nodes That Could Avoid Completion of Axillary Lymph Node Dissection in Operable Breast Cancer
Salmon RJ, de Rieco Y, Falcou MC. Institut Curie, Paris, France.

P3-07-18 Withdrawn

P3-07-19 Long-Term Outcome of Internal Mammary Lymph Node Detected by Lymphoscintigraphy in Early Breast Cancer

P3-07-20 An Independent Assessment of Seven Nomograms for Predicting the Probability of Additional Axillary Nodal Metastasis after Positive Sentinel Lymph Node Biopsy in a Cohort of British Breast Cancer Population
Nadeem RM, Gudder LD, Saldan ZA. Lancashire Teaching Hospitals NHS Foundation Trust, Chorley, United Kingdom.

P3-07-21 Sentinel Lymph Node Metastasis Are More Likely To Develop in Triple Positive Breast Cancer Patients without Compromising Disease Free Survival

P3-07-22 Combined Approach for Staging the Axilla in Breast Cancer Patients with Clinically (-) Nodal Versus Sentinel Node Biopsy Alone
Patel RK, Krol VV, Cibull ML, McGrath PC, Fjallskog M-L, Pirruccello EA, Szabunio AL, Samayoa LM. University of Kentucky, Lexington, KY; Uppsala University, Uppsala, Sweden; VAMC, Lexington, KY.

P3-07-23 Intraoperative Molecular Analysis of Sentinel Lymph Nodes in Breast Cancer Using One Step Nuclease Acid Amplification (OSNA)

P3-07-24 Accuracy and Cost Effectiveness of Frozen Section Examination of the Sentinel Lymph Node (SLN) in Ductal Carcinoma In Situ (DCIS) of Breast
Balleharinna UK, Santoro E, Schaefer SS, Blackwood MM, Chamberlain RS. Saint Barnabas Medical Center, Livingston, NJ; Maimonides Medical Center, Brooklyn, NY; Saint George’s University School of Medicine, West Indies, Grenada; University of Medicine and Dentistry of New Jersey, Newark, NJ.

P3-07-25 Sentinel Lymph Node Mapping in Breast Cancer after Primary Chemotherapy
Bonardi S, Andreis D, Allevi G, Aggugini S, Milani M, Generali D, Bersiga A, Brizzi MP, Dogliotti L, Berruti A, Bottini A. Azienda Ospedaliera Istituti Ospitalieri, Cremona, Italy; Azienda Ospedaliera Universitaria San Luigi di Orbassano, Università di Torino, Orbassano, Torino, Italy.

P3-07-26 How Generalizable Is the Patient Population Enrolled in ACOSOG Z11?
Lannin DR, Killelea BK, Grube BJ, Horowitz N, Chagpar AB. Yale University School of Medicine, New Haven, CT.

P3-07-27 ROCK II Expression Can Be a Potential Marker of Non-Sentinel Lymph Node Metastasis in Breast Cancer Patients with Sentinel Lymph Node Involvement
Shigematsu H, Taguchi K, Shiozumi K, Kawaguchi H, Nishiyama K, Ohno S, Okada M. Hiroshima University Hospital, Hiroshima, Japan; National Kyushu Cancer Center, Fukuoka, Japan.

P3-07-28 One Step Nuclease Acid Amplification (OSNA) for Intraoperative Molecular Detection of Lymph Node Metastases and Micro-Metastases in Breast Cancer
Babar MM, Madani R, Jackson P, Irvine T, Layer G, Kissin M. Royal Surrey County Hospital, Guildford, SU, United Kingdom; University of Surrey, Guildford, SU, United Kingdom.

P3-07-29 Validating the Lymph Node Ratio as a Prognostic Indicator among South East Asian Breast Cancer Patients
Saxena N, Hartman M, Bhoi-Fathy N, Aziz R, Siew EL, Lee SC, Yip CH, Verkooijen HM. National University of Singapore, Singapore; Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia; University Medical Center Utrecht, Utrecht, Netherlands; National University Cancer Institute, National University Health Systems, Singapore, Singapore.

P3-07-30 Applying the Findings of the Z11 Trial to a UK Practice
Chalmers CR, Mallon EA, Stallard S, Doughty JC, Wilson CR. Western Infirmary, Glasgow, Scotland, United Kingdom.

P3-07-31 Intra-Operative Assessment of Sentinel Lymph Nodes in Breast Cancer with Touch Imprint Cytology (TIC) in 460 Consecutive Patients

P3-07-32 The Role of Axillary Ultrasound in the Detection of Metastases from Primary Breast Cancers
Yamashta M, Little E, Fraley J, Hovanessian-Larsen L, Sener SF. Keck School of Medicine, University of Southern California, Los Angeles, CA.
P3-07-33 Are Single Node Metastases More Common in Patients with Breast Cancer in the Sentinel Node Era? 
Shetty G, Randhawa S, Mohammadi I, Hans S, Jones L, Clarke D. 
Warwick Hospital, Warwick, West Midlands, United Kingdom.

P3-07-34 Occult Metastasis in Sentinel Node: Should This Affect the Clinical Decision Making? A Systematic Review and Meta-Analysis

P3-07-35 Validation of Online Calculators To Predict Non-Sentinel Lymph Node Status in Sentinel Lymph Node-Positive Breast Cancer Patients
Tanaka S, Sato N, Fujioka H, Takahashi Y, Kimura K, Iwamoto M. 
Osaka Medical College, Takatsuki, Osaka, Japan.


P3-07-37 Clinical Characteristics and Treatment of Breast Cancer at Public and Private Institutions
Hegg R, Mattar A, Gebrim LH, Emerenciano K, Pinotti M, Perdicaris M, van Eyll B, Franke F, Pinczovskis H, Freitas, Jr R, Jendribova D, Borges G, Queiroz G, Nascimento YY, Gambel O, Mathias C, Budel V, Strepposos E, Delgado G. Perola Byington Hospital, Sao Paulo, SP, Brazil; II. Liga Norte Riograndeense; EGMAJAP, Instituto Santista de Oncologia; Instituto do Cancer Arnaldo Vieira de Canhalho; Hospital de Canadade de Ijuí; Faculdade de Medicina do ABC; Hospital das Clinicas de Goiania, Centro Goiano de Oncologia; Clinica de Neoplasias Litoral; Hospital Araujo Jorge; Cepon, Hospital do Servidor Publico do Estado de Sao Paulo; Nucleo de Oncologia da Bahia; Universidade Federal do Paraná; Hospital Sao Rafael; Hospital Santa Lucinda.

P3-07-38 Selective Omission of Blue Dye in Patients Undergoing Sentinel Lymph Node Biopsy for Breast Cancer
Waters JL, Iqbal M, Jones L, Harries S, Clarke D. Warwick Hospital, South West Warwickshire Foundation Trust, Warwick, West Midlands, United Kingdom.

P3-07-39 Where To Look for Sentinel Lymph Node in Breast Cancer
Durian A. Klinikum Bayreuth GmbH, Bayreuth, Bayern, Germany.

P3-07-40 Impact of the Sentinel Lymph Node Procedure on the Detection of Positive Lymph Nodes in Breast Cancer

P3-07-41 Sentinel Lymph Node Biopsy Is a Reliable Method for Lymph Node Evaluation in Neoadjuvant Chemotherapy Treated Breast Cancer Patients
Kaplan R, Oiu Q, Monni S, Swistel A, Shin S. The New York-Presbyterian Hospital-Weill Cornell Medical Center, New York, NY.

P3-07-42 Lymphovascular Invasion Best Correlates with Presence of Nodal Metastasis in Sentinel Lymph Node Biopsy
Abdiss MS, Adeleka MW, Patel E, Cotrell S. North Manchester General Hospital, Manchester, United Kingdom; University of Manchester, Manchester, United Kingdom.

P3-07-43 The Impact of Timing in Sentinel Lymph Node Biopsy in Primary Breast Cancer
Güth U, Schmid SM, Myrick ME, Oberrmann EC, Viehl CT, Rochlitz C, Forer F. University Hospital Basel, Basel, Switzerland.

P3-07-44 Feasibility of Axillary Reverse Mapping during Sentinel Lymph Node Biopsy in Breast Cancer
Noguchi M, Ohno Y, Nakano Y, Noguchi M, Kosaka T. Kanazawa Medical University, Kahoku, Ishikawa, Japan.

P3-07-45 Role of SPECT-CT in Detecting Sentinel Lymph Nodes in Patients with Ipsilateral Breast Cancer Recurrence and Previous Axillary Lymph Node Dissection

P3-07-46 Accuracy of SPIO-Enhanced MR Imaging Alone for the Diagnosis of Sentinel Node Metastases in Patients with Breast Cancer

P3-07-47 Validated Nomogram To Predict Sentinel Lymph Node Positivity in Breast Cancer Patients

P3-07-48 Axillary Imaging with Dynamic MRI Following Subcutaneous Injection of Superparamagnetic Iron Oxide Nanoparticles
Douek M, Johnson L, Parish J, Charles-Eduards G, Hall-Craggs M. King’s College London, London, London, United Kingdom; Guy’ and St Thomas’ Hospitals, London, United Kingdom; University College Hospital, London, United Kingdom.

P3-07-49 Microinvasive Breast Carcinoma: evaluation of status axillary is necessary?

P3-08-01 Effects of an Integrated Yoga Program on Mood States, Distress, Quality of Life, Diurnal Cortisol Rhythms and Natural Killer Cell Counts in Metastatic Breast Cancer Survivors
Gopinath SK, Rao RM, Sanjeevaavara VH, Diwakar RB, Basavanggila AS, Patil S, Raghuram N, Ramareo N, Ushariam RM. HGC - BIO Super Speciality Centre, Bangalore, Karnataka, India; Swami Vivekananda Yoga Anusandhana Samsthana, Bangalore, Karnataka, India.

P3-08-02 Evaluating the Impact of Educational Material on Anastrozole Treatment Adherence - The Final Results of the ARTEMIS Study
Nogaret J-M, Coibion M, Neven P, Soepenberg O, Graa M-PP, Deschamp V, Vanlereghem T. Jules Bordet Institute, Brussels, Belgium; CHC St-Vincent, Rocourt, Belgium; University Hospital Leuven, Leuven, Belgium; MariaZiekenhuis, Noord-Limburg, Belgium; CHC Saint-Joseph, Liege, Belgium; AstraZeneca Benelux, Brussels, Belgium.

P3-08-03 Exercise Increases Soluble Vascular Endothelial Growth Factor Receptor-1 (sFlt-1) in the Circulation of Adult Women
Makey K, Patterson SG, Robinson J, Loftin M, Waddell DE, Miele L, Chinchar E, Huang M, Smith AD, Weber M, Gu J-W. University of Mississippi Medical Center, Jackson, MS, University of Mississippi, Oxford, MS.

P3-08-04 “How Important is This for Me?” - The Role of Necessity Beliefs as Determinants of Breast Cancer Prevention Intentions among High-Risk Women
Verma S, Paquet L, Stacey D, Davis I, Bedard M, Lowry S, lanni L. The Ottawa Hospital Cancer Centre, Ottawa, ON, Canada; Carleton University, Ottawa, ON, Canada; University of Ottawa, Ottawa, ON, Canada.

P3-08-05 Internet-assisted weight loss for overweight/obese women at high risk for breast cancer
Epidemiology, Risk, and Prevention: Prevention – Nutritional Studies

P3-09-01 Change in Carbohydrate Intake and Breast Cancer Prognosis
Emond JA, Patterson RE, Pierce JP. UCSF Moores Cancer Center, La Jolla, CA; San Diego State University, San Diego, CA.

P3-09-02 Intermittent Dietary Carbohydrate Restriction Enables Weight Loss and Reduces Breast Cancer Risk Biomarkers
Harvie M, Wright C, Pegington M, Mitchell E, Evans DG, Jebb S, Clarke R, Goodacre R, Dunn W, Mattson M, Howell A. University Hospital of South Manchester, Manchester, United Kingdom; MRC Human Nutrition Research Group, Cambridge, United Kingdom; University of Manchester, Manchester, United Kingdom; National Institute of Aging, Baltimore, MD.

P3-09-03 Long-Chain Polysaturated Fatty Acid Intake and Its Relationship to Long-Chain Polysaturated Fatty Acids in Serum, Red Blood Cells and Breast Tissue
Harvey KE, Li S, Carlson SE, Sullivan DK, Klemp JR, Kimler BF, Fabian CJ. University of Kansas Medical Center, Kansas City, KS.

P3-09-04 Comparative Preventive Efficacy of Aqueous Extracts from Lycium Barbarum Bark and Fruit on Estrogen Receptor Positive Human Mammary Carcinoma MCF-7 Cells
Telang NT, Li G, Sepkovic DW, Bradlow LH, Song G, Song G, Telang NT. Palindrome Liaisons, Montvale, NJ; American Institute for Chinese Medicine, New York, NY; Hackensack University Medical Center, Hackensack, NJ.

P3-09-05 Understanding the Role of Estrogen in Sex Differences in Adipocyte Biology for Cancer Prevention
Stubbs RE, Holcomb VB, Hong J, Nunez NP. The University of Texas at Austin, Austin, TX.

P3-09-06 Changes of Serum Vitamin D According to the Breast Cancer Treatment
Kim HJ, Yi OV, Koh BS, Yu JH, Lee JW, Son BH, Ahn SH. Asan Medical Center, Seoul, Korea.

Epidemiology, Risk, and Prevention: Prevention – Preclinical Studies and Model Systems

P3-10-01 Alternative Dosing Regimens with the EGFR Inhibitors (Gefitinib and Lapatinib) in Mammary Cancer Models: Prevention and Therapeutic Efficacy
Lubet RA, Bode AM, Szabo E, Grubbs CJ. National Cancer Institute, Bethesda, MD; Hormel Institute, Austin, MN; University of Alabama at Birmingham, Birmingham, AL.

P3-10-02 Gene Expression Changes in Methylnitrosourea (MNU)-Induced ER+ Mammary Cancers Following Short-Term Treatment of Rats with the Aromatase Inhibitor Vorozole
Lubet RA, Grubbs CJ, Bode A, You M, Lu Y. National Cancer Institute, Bethesda, MD; University of Alabama at Birmingham, Birmingham, AL; Hormel Institute, Austin, MN; Medical College of Wisconsin, Milwaukee, WI.

P3-10-03 Bardoxolone (5MeCDDO) Inhibits Cancer Initiation but Promotes Progression in Rodent Models of Breast Cancer. What Does It Mean for the Antioxidant Response Element (ARE) as a Primary Prevention Target?
Lubet RA, Townsend R, Vedell P, Steele VE, Grubbs CJ. National Cancer Institute, Bethesda, MD; Washington University School of Medicine, Saint Louis, MO; Medical College of Wisconsin, Milwaukee, WI; University of Alabama at Birmingham, Birmingham, AL.

P3-10-04 Halting Early Breast Cancer Progression with Omega-3 Ethyl Esters: Altering Tumor Microenvironment in 21T Series Cell Lines
Chen CH, Rhodes ME, Fabian C, Hursting SD, deGraffenried LA. University of Texas at Austin, Austin, TX; University of Kansas Cancer Center, Kansas City, KS.
P3-12-03  A Prognostic Index of Ipsilateral Breast Tumor Recurrence in Patients Treated with Breast-Conserving Surgery after Preoperative Chemotherapy: Validation of M.D. Anderson Prognostic Index
Ohno S, Ohsumi S, Inaji H, Akiyama F, Akashi-Tanaka S, Sato N, Takahashi K, Dura S. National Kyushu Cancer Center, Fukuoka, Japan; National Shikoku Cancer Center, Matsuyama, Ehime, Japan; Osaka Medical Center for Medical and Cardiovascular Disease, Osaka, Japan; The Cancer Institute of the Japanese Foundation for Cancer Research, Tokyo, Tokyo, Japan; National Cancer Center Hospital, Tokyo, Tokyo, Japan; Niigata Cancer Center Hospital, Niigata, Japan; Shizuoka Cancer Center, Shizuoka, Japan; Wakayama Medical University, Wakayama, Japan.

P3-12-04  Involved Anterior Margins after Breast Conserving Surgery: Is Re-Excision Required?
Mullen R, Macaskill EJ, Khalil A, Elseedawy E, Brown DC, Lee AC, Purdie C, Jordan L, Thompson AM. Ninewells Hospital, Dundee, United Kingdom; Perth Royal Infirmary, Perth, United Kingdom.

P3-12-05  Breast Cancer Recurrence: 2nd Conservative Treatment Versus Mastectomy

P3-13-01  Boost Radiation Therapy Not of Value in Reducing IBTR of Invasive or Noninvasive Breast Cancers for Patients with DCIS: Results from the NSABP B-24 Trial
Julian TB, Vicini FA, Costantino JP, Arthur DW, Kickweil KM, Land SR, Mammounas EP, Wolmark N. National Surgical Adjuvant Breast & Bowel Project, Pittsburgh, PA; Allegheny General Hospital, Pittsburgh, PA; Beaumont Health System, Royal Oak, MI; Virginia Commonwealth University, Richmond, VA; Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA; Ulfman Health Foundation, Canton, OH.

P3-13-02  The Impact of Lymph Node Status on Clinical Outcomes Following Accelerated Partial Breast Irradiation
Shah C, Wilkinson JB, Wallace M, Vicini F. William Beaumont Hospital, Royal Oak, MI.

P3-13-03  Long-Term Symptoms after Radiotherapy of Supracavitary Lymph Nodes in Breast Cancer Patients

P3-13-04  Functional and Cosmetic Outcomes Following Post-Mastectomy Irradiation with Tissue Expander/Implant Reconstruction
Baschnagel A, Shah C, Wilkinson JB, Dekhene N, Margolis J, Arthur DW, Vicini F. William Beaumont Hospital, Royal Oak, MI; VCU Massey Cancer Center, Richmond, VA.

P3-13-05  Analysis of Heart Dose-Volume Parameters and Cardiac Events among Node Positive Breast Cancer (NPBC) Patients Treated with Three-Dimensional Conformal Radiation Therapy (3D-CRT)

P3-13-06  Cardiovascular Magnetic Resonance Imaging and Radiation-Induced Heart Disease Following Radiotherapy for Breast Cancer

P3-13-07  The TARGIT-A Trial Update Confirms No Increase in Local Recurrence
Vaidya JS, Baum M, Wenz F, Bulsara M, Tobias J, Alvarado M, Saunders C, Williams N, Joseph D, on Behalf of the TARGIT Trials Group. University College London, London, United Kingdom; University of Heidelberg, Mannheim, United Kingdom; University of Notre Dame, Fremantle, United Kingdom; University College Hospital and Whittington Hospital, London, United Kingdom; School of Surgery, University of Western Australia, London (All), United Kingdom; University of San Francisco, London (All), United Kingdom; Sir Charles Gairdner Hospital, London (All), United Kingdom.

P3-13-08  Survival Analysis and Recurrence Patterns in Locally Advanced Breast Cancer Following Neoadjuvant Chemotherapy and Preoperative Concurrent Chemo Radiotherapy
Shaw RJ, Lara F, Robles CD, Villar D. Instituto Nacional de Cancerologia, Mexico City, Mexico.

P3-13-09  Impact of Estrogen Receptor Negativity on Clinical Outcomes Following Accelerated Partial Breast Irradiation

P3-13-10  Does Lapatinib Increase Pulmonary Toxicity When Concurrently Used with Radiation Therapy? An Experimental Study with Wistar-Albino Rats

Horowitz DP, Nj J, Burri R. Columbia University Medical Center-New York Presbyterian Hospital, New York, NY.

P3-13-12  Electrons for Intraoperative Breast Radiotherapy in Selected Patients: Long-Term Results of the Montpellier Phase II Trial

P3-14-01  Panitumumab in Combination with FEC 100 (5-Fluorouracile, Epirubicin, Cyclophosphamide) Followed by Docetaxel (T) in Patients with Operable, Triple Negative Breast Cancer (TNBC): Final Results of a Multicentre Neoadjuvant Pilot Phase II Study
Nabholtz J-M, Weber B, Gilgore J, Moutet-Reynier M-A, Tredan H, Tubiana-Mathieu N, Abrial C, Kwiatkowski F, Planchat E, Chalabi N, Penault-Llorca F, Chollet P, Jean Perin Comprehensive Cancer Centre, Clermont-Ferrand, France; National Cancer Centre Hospital, Tokyo, Tokyo, Japan; National Cancer Centre Hospital, Osaka, Japan; The Cancer Institute of the Japanese Foundation, Tokyo, Tokyo, Japan; National Kyushu Cancer Center, Fukuoka, Japan; Niigata Cancer Center Hospital, Niigata, Japan; Shizuoka Cancer Center, Shizuoka, Japan; Wakayama Medical University, Wakayama, Japan.

P3-14-02  The TARGIT-A Trial Update Confirms No Increase in Local Recurrence
Vaidya JS, Baum M, Wenz F, Bulsara M, Tobias J, Alvarado M, Saunders C, Williams N, Joseph D, on Behalf of the TARGIT Trials Group. University College London, London, United Kingdom; University of Heidelberg, Mannheim, United Kingdom; University of Notre Dame, Fremantle, United Kingdom; University College Hospital and Whittington Hospital, London, United Kingdom; School of Surgery, University of Western Australia, London (All), United Kingdom; University of San Francisco, London (All), United Kingdom; Sir Charles Gairdner Hospital, London (All), United Kingdom.
P3-14-02  Sequential Versus Upfront Intensified Neoadjuvant Chemotherapy in Patients with Large Resectable or Locally Advanced Breast Cancer (INTENS), Toxicity Results from a Phase III Study of the Dutch Breast Cancer Trialsists’ Group (BOOG)

Vrins BE, Van de Vijver KK, Boetes C, van Gastel SM, Wals J, Smilde TJ, van Warmerdam LJ, van Laarhoven HW, van Spriens DJ, Born GF, Tjan-Heijnen VC. Maastricht University Medical Centre, Maastricht, Netherlands; Comprehensive Cancer Centre the Netherlands, Nijmegen, Netherlands; Atsum Medical Centre, Heerenlen, Netherlands; Jeroen Bosch Hospital, ’s Hertogenbosch, Netherlands; Catharina-Hospital, Eindhoven, Netherlands; Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands; Canisius-Wilhelmina Hospital, Nijmegen, Netherlands.

P3-14-03  ABCB1 Single Nucleotide Polymorphisms a Possible Prognostic Factor in Breast Cancer Patients Receiving Docetaxel and Doxorubicin Neoadjuvant Chemotherapy on Systemic Treatment


P3-14-04  Assessment of Genomic Prognostic Signatures as Predictors of Response to Neoadjuvant Chemotherapy in Patients with Early Stage Breast Cancer

Culakova E, Poniewierski MS, Huang M, Kuderer NM, Ginsburg S, Barry W, Marcom PK, Ready N, Abernethy A, Lyman GH. Duke University, Durham, NC.

P3-14-05  Evaluation of Residual Cancer Burden Index (RCBI) as a Predictor of Disease Free Survival in a Non-Selected Cohort of Breast Cancer Patients Treated in the Neoadjuvant Setting

Perez-Fidalgo J-A, Martinez M, Ferrer J, Pons V, Bermejo B, Furriol J, Eroles P, Lluch A. Hospital Clinic, Barcelona, Spain; Hospital Arnau de Vilanova, Lleida, Spain; University of Lleida, Barcelona, Spain; Luisenkrankenhaus Düsseldorf, Düsseldorf, Germany; Ospedale Sacro Cuore, Negrar (Verona), Italy; Cliniques Universitaires St-Luc, Université Catholique de Louvain, Brussels, Belgium; Technischen Universität München, München, Germany; Centre Aexitis Vaurini, Vandoeuvre-Les-Nancy, France; Cephalon, Maisons-Alfort, France; Frauenklinik und Brustzentrum Rheinfelden, Rheinfelden, Germany.

P3-14-06  Combined Use of 18F-FDG PET/CT and MRI To Monitor Breast Cancer Response during Neoadjuvant Chemotherapy

Pengel KE, Koolen BB, Vogel W, Valdes Olmos R, Wesseling J, Vanrenck Peeters M-J, Loc CE, Higuys KG. Netherlands Cancer Institute, Amsterdam, Netherlands; Netherlands Cancer Institute, Amsterdam; University Medical Center Utrecht, Utrecht, Netherlands.

P3-14-07  Relative Risk of Recurrence (RR) over Time in ER Positive and Negative T4 Breast Cancer Patients Achieving Less Than pCR (<pCR) after Primary Chemotherapy: A Reversal Trend of Recurrence beyond 60 Months after Diagnosis

Ionta MT, Atzoni F, Pusceddu V, Notar F, Valle G, Guarzoni D, Chiappe M, Marangiu M, Minervia L, Massidda B. University Hospital, Cagliari, Italy; Hospital of Oncology Buisinco, Cagliari, Italy.

P3-14-08  A Phase II Study of Gemcitabine and Carboplatin (GC) Plus Iniparib (BSI-201) as Neoadjuvant Therapy for Triple-Negative and BRCA1/2 Mutation-Associated Breast Cancer


P3-14-09  A Phase II Preoperative Study of Dasatinib, a Multi-Targeted Tyrosine Kinase Inhibitor, in Locally Advanced “Triple-Negative” Breast Cancer Patients

Rimawi MF, Rodriguez AA, Yang WT, Gonzalez-Angulo AM, Nangia JR, Wang T, Speers C, Mills G, Hilsenbeck SG, Brown PH, Chang JC. Baylor College of Medicine, Houston, TX; The Methodist Hospital, Houston, TX; M.D. Anderson Cancer Center, Houston, TX.

P3-14-10  Early Predictive Value of Non-Responder to Docetaxel in Neoadjuvant Chemotherapy in Breast Cancer Using 18F-FDG-PET

Hirakata T, Fujisawa T, Yanaigiti H, Hiyoshi K, Oya N, Akyoishi T, Kinoshita T, Kuvano H. Gunma Prefectural Cancer Center, Ota, Gunma, Japan; Tsursuyaga Hospital, Isesaki, Gunma, Japan; Graduate School of Medicine, Gunma University, Maebashi, Gunma, Japan.

P3-14-11  Comparison of Two Nomograms To Predict Pathologic Complete Response to Neoadjuvant Chemotherapy - Evidence That HER2 Positive Tumors Need Specific Predictors


P3-14-12  Local Control of Primary Breast Cancer Treated with Radical Radiotherapy Alone after Neoadjuvant Chemotherapy

Makris A, Li SP, Ravichandran D, Ostler PJ, Pittman M. Mount Vernon Cancer Centre, Northwood, Middlesex, United Kingdom; Luton and Dunstable, Luton, United Kingdom.

P3-14-13  A Prospective Open-Label Randomized Phase II Neoadjuvant Study of Non-Pegylated Liposomal Doxorubicin (MYOCET®) Plus Cyclophosphamide and Trastuzumab Versus Conventional Doxorubicin Plus Cyclophosphamide Alone, Each Followed by Docetaxel and Trastuzumab, in HER2-Positive Breast Cancer Patients

Llombart-Cussac A, Perrin-Simon S, Rezai M, Hauschild M, Venturini M, Machels J-P, Paepe S, Luporsi E, Kasiskorski F, Kayaline L. Hospital Amava de Vilanova, Lleida, Spain; Hospital T’s Hospitalet de Llobregat, Barcelona, Spain; Luisenkrankenhaus Düsseldorf, Düsseldorf, Germany; Ospedale Sacro Cuore, Negrar (Verona), Italy; Cliniques Universitaires St-Luc, Université Catholique de Louvain, Brussels, Belgium; Technischen Universität München, München, Germany; Centre Aexitis Vaurini, Vandoeuvre-Les-Nancy, France; Cephalon, Maisons-Alfort, France; Frauenklinik und Brustzentrum Rheinfelden, Rheinfelden, Germany.

P3-14-14  Increased Prevalence of Low Vitamin D Level in Breast Cancer Patients during Neoadjuvant Chemotherapy


P3-14-15  Non-Randomized, Open Label Phase II Trial Evaluating the Safety and Efficacy of Taxotere (T) Followed by Myocet (M) + Cyclophosphamide (C) as First-Line Treatment for HER2-Negative Breast Cancer (BC)

Garcia-Mata J, Calvo L, Lopez R, Ramos M, Castellanos J, Heras L. Complexo Hospitalario de Ourense, Ourense, Spain; Complexo Hospitalario Universitario A Coruña, A Coruña, Spain; Hospital Clinico de Santiago, Santiago de Compostela, A Coruña, Spain: Complexo Hospitalario Universitario Xeral Cies, Vigo, Pontevedra, Spain; Hospital Sociosanitari de L’Hospitalet, Barcelona, Spain.

P3-14-16  Molecular Phenotype and the Use of HER-2 Targeted Agents Influence the Accuracy of Breast MRI after Neoadjuvant Chemotherapy


P3-14-17  Paclitaxel, Carboplatin, and Trastuzumab in a Neoadjuvant Regimen for HER2-Positive Breast Cancer: The TRAIN Study

Sonke GS, Barry W, Marcom PK, Ready N, Abernethy A, Lyman GH. Duke University, Durham, NC; BiPar Sciences, South San Francisco, CA.

P3-14-18  Primary Tumor Response to Neoadjuvant Chemotherapy Is Significantly Associated with Nodal Pathological Complete Response in Breast Cancer Patients with Cytologically Proven Axillary Node Metastasis

Hwang H, Park S, Lee JS, Kim S-J, Park B-W. Yonsei University College of Medicine, Seoul, Korea.
P3-14-19 Impact of Chemotherapy-Induced Amenorrhea on Response to Neoadjuvant Chemotherapy in Breast Cancer
Ahn SK, Moon HG, Kim JS, You JM, Shin Hc, Han W, Noh D-y. Seoul National University Hospital.

P3-14-20 Concomitant Taxane-Anthracyclin Regimen for Neoadjuvant Chemotherapy of Primary Breast Cancer: Experience from a Cohort of 223 Patients Treated in a Single Institution

P3-14-21 Neoadjuvant Therapy Response, Subtype and BRCA Status in an Underserved Population

P3-14-22 Response to Neoadjuvant Chemotherapy in Elderly Patients with Locally Advanced Breast Cancer

P3-14-23 Response to Neoadjuvant Chemotherapy and Survival in Japanese Patients with Triple-Negative Breast Cancer
Ohtani S, Kochi M, Ito M, Takada S, Matsuura H, Higaki K. Hiroshima City Hospital, Hiroshima, Japan.

P3-14-24 A Phase II Trial of TS-1 and Docetaxel Followed by 5-FU/Epirubicin/Cyclophosphamide (FEC) as Preoperative Treatment in Women with Stage II/III Breast Cancer
Hayashida T, Jinno H, Sakata M, Takahashi M, Kitagawa Y. Keio University School of Medicine, Tokyo, Japan.

P3-14-25 Neoadjuvant Trastuzumab and Paclitaxel Combination Increases a High Rate of Pathological Complete Responses in Locally Advanced Breast Cancer by Exploiting Host Antitumor Immunity

P3-14-26 The Effect of Biologic Subtype in Patients Treated with Neoadjuvant Chemotherapy: A UAB Experience
Keene KS, De Los Santos JF, Meredith R, Hinton B, Li Y, Krontritas H, Bland K, Carpenter JT, Forero A. University of Alabama at Birmingham, Birmingham, AL.

P3-14-27 Pegylated Liposomal Doxorubicin (PLD) as Primary Treatment in Estrogen Receptor (ER) and HER2 Poor Breast Cancer and Risk of Developing Cardiotoxicity or Elderly Patients (pt).

P3-14-28 ANZ 0502 NeoGem: A Phase II Trial Evaluating the Efficacy and Safety of Epirubicin and Cyclophosphamide Followed by Docetaxel with Gemcitabine (+ Trastuzumab If HER2 Positive) as Neoadjuvant Chemotherapy for Women with Large Operable or Locally Advanced Breast Carcinoma
McNulty N, Boyle B, Bull J, Leong E, Simpson A, Kannouakis G, Gebski V, Forbes JF, Wolkens N, Lindsay DF, Badger HD. Royal Brisbane and Women’s Hospital, Brisbane, Queensland, Australia; The Mater Hospital, Sydney, New South Wales, Australia; Wellington Hospital, Wellington, New Zealand; Ballarat Oncology and Haematology Service, Ballarat; Victoria, Australia; NHRMC Clinical Trials Centre, Sydney, New South Wales, Australia; University of Newcastle, Newcastle, New South Wales, Australia; Calvary Mater Newcastle, Newcastle; New South Wales, Australia; Westmead Hospital, Sydney, New South Wales, Australia; Australian New Zealand Breast Cancer Trials Group, Newcastle, New South Wales, Australia.

P3-14-29 Neoadjuvant Sunitinib Administered with Weekly Paclitaxel/Carboplatin in Patients with Locally Advanced Triple-Negative Breast Cancer: A Sarah Cannon Research Institute Phase II/II Trial

P3-14-30 Concurrent Celescoxi with FEC Followed by Docetaxel Shows Good Responses and Prognosis in a Neoadjuvant Breast Cancer Study
Chow LWC, Tung SY, Ng T-Y, Oh D-Y, Im S-A, Lee M-H, Yip AYS, Toi M, Glück S. Organisation for Oncology and Translational Research, Hong Kong; UNIMED Medical Institute, Hong Kong; Tuen Mun Hospital, Hong Kong; Seoul National University Hospital, Seoul, Korea; Soochunhyang University Hospital, Seoul, Korea; Kyoto University Hospital, Kyoto, Japan; University of Miami Leonard M. Miller School of Medicine, Miami, FL.

P3-14-31 Preoperative Chemotherapy and Bevacizumab for Locally Advanced HER-2 Negative Breast Cancer Followed by Prolonged Postoperative Bevacizumab for Those with Less Than Complete Pathologic Response
Carpenter JT, Forero A, Falkson CI, Nabeil LM, De Los Santos JF, Krontritas H, Bland K, Li Y. University of Alabama at Birmingham, Birmingham, AL.

P3-15-01 An Interim Efficacy Analysis of Neoadjuvant Letrozole in the New Primary Endocrine-Therapy Origination Study (NEOS/N-SAS BC06): A Randomized Study of Adjuvant Endocrine Therapy with or without Chemotherapy for Postmenopausal Breast Cancer Patients Who Responded to Neoadjuvant Letrozole
Iwata H, Yamaguchi T, Masuda N, Toyama T, Kashiwaba M, Yamamoto Y, Taia N, Saji S, Ohashi Y. Aichi Cancer Center, Nagoya, Japan; Tohoku University Graduate School of Medicine, Sendai, Japan; NHO Osaka National Hospital, Osaka, Japan; Nagoya City University Hospital, Nagoya, Japan; Iwate Medical University, Morioka, Japan; Kumamoto University Hospital, Kumamoto, Japan; Okayama University Hospital, Okayama, Japan; Satama Medical University International Medical Center, Hidakura, Japan; The University of Tokyo, Tokyo, Japan.

P3-15-02 The Change of Bone Turnover Markers during Neoadjuvant Anastrozole Versus Exemestane: A Randomized Single-Center Study
Jinno H, Shima K, Takahashi M, Hayashida T, Hirose S, Ikekda T, Kitagawa Y. Keio University School of Medicine, Shinjuku, Tokyo, Japan; Teikyo University School of Medicine, Itabashi, Tokyo, Japan.
P3-16-01  Safety Profile and Clinical Activity of Single-Agent BKM120, a Pan-Class I PI3K Inhibitor, for the Treatment of Patients with Metastatic Breast Carcinoma

Rodon J, Bendell JC, Abdul Razak AR, Homji N, Trandafir I, Quadt C, Graña-Suárez B, Siu LL, Di Tomaso E, Demanet D, Massacesi C, Hirawat S, Burns III HA, Baselga J. Vall d’Hebron University Hospital, Barcelona, Spain; Sarah Cannon Research Institute, Nashville, TN; Princess Margaret Hospital, Toronto, ON, Canada; Novartis Pharmaceuticals, Florham Park, NJ; Novartis Oncology, Paris, France; Novartis Pharma AG, Basel, Switzerland; Novartis Institutes for BioMedical Research Inc, Cambridge, MA, Massachusetts, Boston, MA.

P3-16-02  Targeting Tumor Initiating Cells with siRNA/Nanotherapy in Triple Negative Breast Cancer

Granados-Principal S, Deng X, Liu D, Chang JC, Shen H, Dave B. The Methodist Research Institute, Houston, TX; Baylor College of Medicine, Houston, TX.

P3-16-03  Enhancing Anti-IGF Directed Therapeutics by Co-Targeting Autophagy

Oh AS, LaPara K, Yee D. University of Minnesota, Minneapolis, MN.

P3-16-04  A Novel Monoclonal Antibody to Secreted Frizzled Related Protein 2 Inhibits Triple Negative Breast Carcinoma Growth Rate In Vivo


P3-16-05  A Phase II Trial Expansion Cohort of the PARP Inhibitor Veliparib (ABT888) and Temozolomide in BRCA1/2 Associated Metastatic Breast Cancer

Isakkoff SJ, Overmoyer B, Tung NM, Gelman RS, Habin K, Qian J, Giranda V, Shepherd S, Garber JE, Ellisien LW, Winer EP, Goss PE. Massachusetts General Hospital Cancer Center, Boston, MA; Dana Farber Cancer Institute, Boston, MA; Beth Israel Deaconess Medical Center, Boston, MA; Harvard Medical School, Boston, MA; Abbott Laboratories, Abbott Park, IL.

P3-16-06  Phase II Trial of T-1 in Combination with Oxaliplatin (SOX) in Patients with Metastatic Breast Cancer (MBC) Previously Treated with Anthracycline and Taxane Chemotherapy

Im S-A, Oh D-Y, Lee KS, Ahn J-H, Shin J, Ahn JS, Kim JH, Han SW, Lee MH, Lee KE, Lee K, Kim HJ, Keam B, Kim S-Y, Kim SB, Im YH, Ro J, Park H-S. Seoul National University Hospital, Seoul, Republic of Korea; National Cancer Center, Republic of Korea; Asan Medical Center, Seoul, Republic of Korea; YonSei University College of Medicine, Severance Hospital, Seoul; Samsung Medical Center, Seoul, Republic of Korea; Seoul National University Bundang Hospital, Seongnam, Republic of Korea; Inha University Hospital, Incheon, Republic of Korea; Ewha Womans University Medical Center, Seoul, Republic of Korea; Hanlim University Pyungchon Hospital, Pyungchon, Republic of Korea; Korea University; Kyung Hee University Hospital, Seoul, Republic of Korea; Soon Chun Hyang University Hospital, Seoul, Republic of Korea.

P3-16-07  Effect of Denosumab Treatment for Patients with Breast Cancer and Bone Metastases: Results from the Open-Label Extension Treatment Phase

Stopeck AT, Lipton A, Martin M, Body J-L, Paterson A, Steger GG, Tonkin K, De Boer RH, Fujiiwara Y, Yardley D, Jassem J, Takano T, Solal-Celigny P, Fan M, Braun A. Arizona Cancer Center, University of Arizona, Tucson, AZ; Penn State Milton S. Hershey Medical Center, Hershey, PA; Hospital General Universitario Gregorio Marañón, Madrid, Spain; CHU Brugmann, Université Libre de Bruxelles, Brussels, Belgium; Tom Baker Cancer Centre, Calgary, AB, Canada; Medical University of Vienna, Vienna, Austria; Cross Cancer Institute, Edmonton, AB, Canada; Royal Melbourne and Western Hospitals, Melbourne, Australia; National Cancer Center Hospital, Tokyo, Japan; Sarah Cannon Research Institute, Nashville, TN; Tennessee Oncology, PLLC, Nashville, TN; Akademickie Centrum Kliniczne Szpital Akademicki Medycznej w Gdaniku, Gdansk, Poland; Toranomon Hospital, Tokyo, Japan; Clinique Victor Hugo, Centre Jean Bernard, Le Mans, France; Argen, Inc., Thousand Oaks, CA.

P3-16-08  A Phase 2, Randomized Open-Label Study of Iniparib, Administered Either Weekly or Twice-Weekly in Combination with Gemcitabine Plus Carboplatin in Patients with mTNBC

Diéras V, Bonnefoi H, Alba E, Awada A, Coudert B, Pivot X, Gilgorev J, Jäger A, Giannini L, Lindeman G, Pham N, Su Y, Gao M, Mery-Mignard D, Pardiens R, Verweij J. Institut Curie, Paris, Cedex 05, France; Université de Bordeaux, Bordeaux, INSERM, France; Hospital Clínico Universitario Virgen de la Victoria, Málaga, Spain; Institut Jules Bordet, Centre des Tumeurs de l’Université Libre de Bruxelles, Brussels, Belgium; Centre Georges François Leclerc, Dijon, France; University Hospital Jean Minjoz, Besançon, France; University Paris VI, Paris, France; Erasmus University Medical Center, Daniel den Hoed Cancer Center, Rotterdam, Netherlands; HSR - San Raffaele, Milan, Italy; The Royal Melbourne Hospital and The Walter and Eliza Hall Institute of Medical Research, Melbourne, Victoria, Australia; Sanofi, Vitry, Paris, France; Sanofi, Great Valley, PA; Sanofi, Cambridge, MA; University Hospital Gasthuisberg, Catholic University of Leuven, Leuven, Belgium; Erasmus University Medical Center, Rotterdam, Netherlands.

P3-16-09  Endoxifen, a Newly Developed Breast Cancer Drug, Has Anabolic Actions on the Mouse Skeleton


P3-16-10  The Efficacy of Zoledronic Acid in Breast Cancer Adjuvant Therapy: A Meta-Analysis of Randomized Controlled Trials

Lu J, Yan Y, Zhui Q, Zhou L, Jiang Y, Diu Y, Shao Z. Fudan University Shanghai Cancer Center, Shanghai, China; Shanghai Medical College, Fudan University, Shanghai, China.

P3-16-11  Prospective Evaluation of Radiation Pneumonitis in Neoadjuvant Concurrent Docetaxel and Radiation Therapy for Locally Advanced Breast Cancer


P3-16-12  Cardiac Safety of Non-Pegylated Liposomal Doxorubicin and Docetaxel as 1st Line Treatment in Metastatic HER2 Negative Breast Cancer (Myotax Study)


P3-16-14  Effect of TG02, a Multikinase Inhibitor, on Triple Negative Breast Cancer Cells

Pandirri A, Ortiz-Ruiz MJ, Burrows F, Ocaña A, Espiria-Ogando A. Cancer Research Center, Salamanca, Spain; Hospital Universitario Albacete, Spain; Tragara Pharmaceuticals, San Diego, CA.
P3-16-15  Phase I Study with Biomarker Evaluation of Neoadjuvant Sunitinib in Combination with Exemestane in Post-Menopausal Women with Hormone-Sensitive, Her-2 Negative Primary Breast Cancer
Morales S, Gil M, Lломbart A, Garcia M, Uruticoechea A, Pernas S. University Hospital Arnau de Vilanova, Lleida, Cataluña, Spain; University Hospital ICO-Duran i Reynals, Barcelona, Cataluña, Spain.

P3-16-16  Overcoming EGFR Resistance Using Dasatinib in Combination with Cetuximab and Cisplatin in Triple Negative Breast Cancer Cell Lines
Kim EMH, Gartner E, Choi L, Boerner J. Wayne State University, Karmanos Cancer Center, Detroit, MI.

P3-16-17  The Identification of Novel Microtubule Stabilizing Taconalolides
Risinger AL, Li J, Peng J, Chen Z, Mooberry SL. University of Texas Health Science Center, San Antonio, TX; Shanghai Institute of Materia Medica, Shanghai, China.

P3-16-18  Phase 2, Open-Label Study of EZN-2208 (PEG-SN38) in Patients with Previously TreatedMetastatic Breast Cancer
O’Shaughnessy JA, Osborne CRC, Steinberg MA, Holmes FA, Kim HS, Kocs DM, Richards PD, Vukelja SJ, Berkowitz N, Buchbinder A. Texas Oncology - Baylor Charles A. Sammons Cancer Center, Dallas, TX; Virginia Oncology Associates, Norfolk, VA; Texas Oncology - Houston Memorial City, Houston, TX; Rocky Mountain Cancer Centers, Denver, CO; Texas Oncology - Austin Midtown, Austin, TX; Oncology & Hematology Associates of Southwest Virginia, Inc., Danville, VA; D.A. Blue Ridge Cancer Care, Salem, VA; Tyler Cancer Center, Tyler, TX; US Oncology; Enzon Pharmaceuticals, Inc., Piscataway, NJ.

Treatment – Therapeutic Strategies: Novel Drugs and Targeted Agents

P3-17-01  ApoE and Its Receptors (LRP8, VLDLR) Function as Growth Signals for Triple-Negative Breast Cancer and Represent a Novel Therapeutic Target

P3-17-02  Targeting the Autophagy Pathway for Drug Resistance of Breast Tumor-Initiating Cells
Zhao H, Li F, Cui K, Sheng J, Landis M, Chang J, Wong S, Dave B. The Methodist Hospital Research Institute, Weill Medical College, Cornell University, Houston, TX; The Methodist Hospital, Weill Medical College, Cornell University, Houston, TX.

P3-17-03  ADAM17: A Novel Therapeutic Target for Treatment of Triple Negative Breast Cancer
McGowan PW, Mulloylo M, Sukor S, Madden S, McDermott E, Pierce A, Crown J, O’Donovan N, Duffy MJ. St. Vincent’s University Hospital, Dublin, Ireland; University College Dublin, Dublin, Ireland; Dublin City University, Dublin, Ireland; Molecular Therapeutics Cancer Ireland, Dublin, Ireland.

P3-17-04  CKCR1/2 Regulates Human Breast Cancer Stem Cell Activity Via EGFR/HER2-Dependent and -Independent Pathway
Singh JK, Farnie G, Clarke RB, Bundred NJ. School of Cancer and Women’s Health, University of Manchester, Paterson Institute for Cancer Research, Manchester, United Kingdom; University Hospital of South Manchester, Wythenshawe Hospital, Manchester, United Kingdom.

P3-17-05  Beyond HER2 and Hormonal Agents: The Heat Shock Protein 90 Inhibitor Ganetespib as a Potential New Breast Cancer Therapy

P3-17-06  Final Results of a Controlled, Randomized 3-Arm Phase II Trial of EndoTAG™-1, a Cationic Liposomal Formulation of Paclitaxel Targeting Tumor Endothelial Cells, in Advanced Triple-Negative Breast Cancer (TNBC)
Awada A, Bondarenko IN, Tarasova O, Bonnetere J, Nowara E, Ferrero JM, Bakshi AV, Weidenthaler H, Wilke C, Piscart MJ. Institut Jules Bordet, Brussels, Belgium; Dnepropetrovsk State Medical Academy, Dnepropetrovsk, Ukraine; Academy of Medical Sciences of Ukraine, Kharkov, Ukraine; Centre Oscar Lambret, Lille, France; Instytut im. M.Skdowskiej-Curie, Gliwice, Poland; Centre Antoine Lacassagne, Nice, France; Kaushalya Medical Foundation, Thane, India; Medigene AG, Martinsried, Germany.

P3-17-07  EGCG, a Green Tea Antioxidant Suppresses Breast Tumor Angiogenesis and Growth Via Inhibiting the Activation of HIF-1α and NFκB, and VEGF Expression
Makey KI, Tucker KB, Chinchir E, Miele L, Pei I, Thomas EY, Gu J-W. University of Mississippi Medical Center, Jackson, MS.

P3-17-08  Macroautophagy Protects Breast Cancer MCF-7 Cells from TAM-Induced Apoptosis Via Mitogen-Activated Protein Kinase (MAPK) Pathway
Hou YF, Ma XY, Liu Z, Yu SJ, Shao ZM. Cancer Institute, Shanghai, China.

P3-17-09  Neutralizing the Prolactin Receptor with Therapeutic Antibody LFA102: A Novel Approach for the Treatment of Breast Cancer

P3-17-10  EpCAM as a Target for Chemical Antibodies in Metastatic Breast Cancer
Shigdar SL, Du W. Deakin University, Geelong, Victoria, Australia.

P3-17-11  Dovitinib (TKI258), a Dual Inhibitor of FGFR and VEGFR, Induces Tumor Growth Suppression in Xenograft Models of Primary Human Breast Cancer
Shi WM, Linnartz R, Versace R, Graus Porta D, Kay A, Dugan M, Novartis Oncology, East Hanover, NJ; Novartis Institutes for BioMedical Research, Basel, Switzerland.

P3-17-12  Regulation of tubulin detyrosination—a cancer cell specific antimicrotubule strategy for breast cancer treatment
Bleys SW, Sung Y-NS, Hall MD. Sahab ZI. Georgetown-Lombardi Comprehensive Cancer, Washington, DC.

P3-17-13  Romidepsin inhibits growth and metastasis and is synergistic with paclitaxel in pre-clinical models of inflammatory breast cancer
Robertson FM, Chu K, Mu Z, Boyle KM, Green TL, Moraes R, Zhang X, Alpaugh RR, B Photography A, Ye Z, Wright MC, Liu H, Luo AZ, Barsky SH, Heise C, Cristofanilli M. The University of Texas MD Anderson Cancer Center, Houston, TX; , Fox Chase Cancer Center, Philadelphia, PA; University of Nevada School of Medicine and Nevada Cancer Center, Reno and Las Vegas, NV, Celgene Corporation, San Francisco, CA.

Treatment – Therapeutic Strategies: Signal Transduction Inhibitors

P3-18-01  cMET Inhibitor and the Inhibition of Growth of Breast Cancer Cells in Bone Marrow Matrix Environment
Ye L, Mazon MD, Bramble P, Jiang WG. Cardiﬁ University School of Medicine, Cardiff, Wales, United Kingdom; Astra-Zeneca Pharmaceuticals, Surr, United Kingdom.

P3-18-02  A Combination of Pathway-Targeted Inhibitor with DNA-Repair Inhibitor: Preclinical Efficacy of Zsamtima and Olaparib in Triple Negative Subset of Breast Cancer
Dey N, Wu H, Sun Y, De P, Leyland-Jones B. Emory University, Atlanta, GA.
P3-18-03  In Vitro Potency of mTOR Kinase (TOR1/TOR2) Inhibitor, INK128 in ER+ and HER2 Overexpressing Breast Cancer Cells
De P, Sun Y, Dey N, Leyland-Jones B. Emory University, Atlanta, GA

P3-18-04  Pathway Guided Selection of Targeted Inhibitors for Breast Cancer Treatment

P3-18-05  Triple-Negative Breast Cancer: Stem Cells, Cancer and New Treatment Strategy
Yin S, Xu L, Reddy KB. Wayne State University School of Medicine, Detroit, MI

Engel J, Sospelt I, Honig A, Hahne JC, Teifel M. Medical University of Würzburg, Würzburg, Germany; Attema Zentaris GmbH, Frankfurt, Germany.

P3-18-07  Multiplex RTK Inhibitor Screening Utilizing a Plate-Based Immunoassay with Near-Infrared Detection
Finkel DJ, James AE, Felix RA, Wegner GI. R&D Systems Inc., Minneapolis, MN; Tecnos Bioscience, Bristol, United Kingdom.

5:15 pm–7:00 pm POSTER DISCUSSION V: HER2

Ballroom A

Viewing 5:15 pm
Discussion 5:30 pm
Discussant: Michael F. Press, MD, PhD
USC/ Norris Comprehensive Cancer Center
Los Angeles, CA

PD05-01  Trans-CHER-Lob: A Biomarker Analysis of the Randomized Phase II Study of Neoadjuvant Chemotherapy Plus Trastuzumab, Lapatinib or Combined Trastuzumab and Lapatinib in HER2 Positive Operable Breast Cancer
Guameri V, Frassoldati A, Faciara G, Maiorana A, Bettelli S, Bottini A, Cagossi K, Bisagni G, Ravaoli A, Amadori D, Musolino A, Cavanna L, Orlando L, Giardina G, Piacentini F, Bagnalata M, Conte P. Modena University Hospital, Modena, Italy; Medical Oncology, Ferrara University Hospital; Modena University Hospital; Istituti Ospitalieri, Cremona; Ramazzini Hospital, Carpi; Arcispedale Santa Maria Nuova, Reggio Emilia; Ospedale Infermi, Rimini; Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori, Meldola (FC); Parma University Hospital; Hospital of Piacenza; Ospedale di Circolo e Fondazione Macchi, Varese; Perrino Hospital, Brindisi; GlaxoSmithKline, Verona.

PD05-02  Effect of HER2/Topoisomerase II alpha (TOP2A) Gene Status on Protein Expression and Chromosome 17 (CEP17) Polysomy on the Outcome of Breast Cancer Patients Treated with Anthracycline-Containing Dose-Dense Sequential Adjuvant Chemotherapy with or without Paclitaxel - A Pooled Analysis of Two Hellenic Cooperative Oncology Group (HeCOG) Phase III Trials

PD05-03  Impact of Quantitative Measurement of HER2, HER3, HER4, EGFR, ER and PTEN Protein Expression on Benefit to Adjuvant Trastuzumab in Early-Stage HER2+ Breast Cancer Patients in NCTCG N9831
Perez EA, Ballman KV, Reinholz MM, Dueck AC, Cheng H, Jenkins RB, McCullough AE, Chen B, Davidson NE, Martino S, Kaufman PA, Kutteh LA, Sledge GW, Geiger XJ, Ingle JN, Tenner KS, Harris LN, Gralow JR, Rimm DL. Mayo Clinic, Jacksonville, FL; Mayo Clinic, Rochester, MN; Mayo Clinic, Scottsdale, AZ; University of Pittsburgh Cancer Institute, Pittsburgh, PA; Angeles Clinic and Research Institute, Santa Monica, CA, Dartmouth Hitchcock Medical Center, Lebanon, NH, Oncology Associates of Cedar Rapids, Cedar Rapids, IA, Indiana University Medical Center Cancer Pavilion, Indianapolis, IN; Yale University, New Haven, CT; Seattle Cancer Care Alliance, Seattle, WA.

PD05-04  Quantitative Measurement of Antigen Degradation in NCTCG N9831 Tissue Microarrays
Cheng H, Rimm DL, Reinholz MM, Lingle WL, Ballman KV, Dueck AC, Chen B, McCullough AE, Jenkins RB, Perez EA. Yale University School of Medicine, New Haven, CT; Mayo Clinic, Rochester, MN; Mayo Clinic, Scottsdale, AZ; Mayo Clinic, Jacksonville, FL.

PD05-05  Meta Analysis of Discordant HER2 Status in Matched Primary and Metastatic Breast Cancer
Richter S, Zee A. Princess Margaret Hospital; University of Western Ontario.

PD05-06  Determination of HER2 Status with Analysis of Plasma DNA by Digital PCR in Patients with Metastatic Breast Cancer
Greaser MK, Sevensliewen H, Smith E, Ashworth A, Turner NC, Institute of Cancer Research, London, United Kingdom; Royal Marsden Hospital, London, United Kingdom.

PD05-07  Prospective Validation and Characterization of HER2 Positive Circulating Tumor Cells in Patients with HER2 Negative Metastatic Breast Cancer

5:15 pm–7:00 pm POSTER DISCUSSION VI: COST EFFECTIVENESS/UTILIZATION

Ballroom B

Viewing 5:15 pm
Discussion 5:30 pm
Discussant: Thomas J. Smith, MD, FACP
Johns Hopkins
Baltimore, MD

PD06-01  Molecular Classification with 21 Gene Assay (OncoType Dx) in 196,967 ER Positive Patients Shows High Frequency of Low Recurrence Score (LRS) in Both Node Positive (N+) and Negative (N-) Breast Cancer (BrCa) Cohorts. Cost and Guideline Implications

PD06-02  Cost-Effectiveness Evaluation of the Oncotype DX® Breast Cancer Assay in Clinical Practice in the UK
Holt SDH, Bennett H, Bertelli G, Valentine WJ, Phillips CJ. Prince Philip Hospital, Llanelli, United Kingdom; Cardiff Research Consortium, Cardiff, United Kingdom; Singleton Hospital, Swansea, United Kingdom; Ossian Health Economics and Communications, Basel, Switzerland; Swansea University, Swansea, United Kingdom.

PD06-03  Cost Effectiveness Analysis of BRCA1/2 Genetic Testing
Li Q, Holland M, Huston A, Noyes K. University of Rochester School of Medicine, Rochester, NY.
OT2-01-03 Eribulin in Women with Locally Advanced Breast Cancer Who Do Not Achieve Pathologic Complete Response (pCR) Following Neoadjuvant Chemotherapy: A Sarah Cannon Research Institute Phase II Trial
Yardley DA, Peacock NW, Hainsworth JD, Burris III HA. Sarah Cannon Research Institute, Nashville, TN. Tenesse Oncology, PLLC, Nashville, TN.

OT2-01-04 Cardiac Safety of Anthracycline-Containing Adjuvant Chemotherapy of Early Breast Cancer: OSCAR/ABC Ongoing, Observational, Multicentric Study
Ricevuto E, Cociolone V, Zilli M, Scognamiglio MT, Pistilli B, Di Menna G, Mancini M, Carnita K, Adinolfi MI, Ferrandina MG, Piccotti A, Recchia F, Latini L, Ficorella C, Iacobelli S. San Salvatore Hospital, University of L'Aquila, L'Aquila, Italy; SS. Annunziata Hospital and G. Bembo Hospital, University G. D'Annunzio, Chieti, Italy; Ospedale Civile di Macerata, Macerata, Italy; F. Renzetti Hospital, Lanciano, Italy; Catholic University of the Sacred Heart, Rome, Campobasso, Italy; Mazzini Hospital, Teramo, Italy; Ospedale Civile di Avezzano, Avezzano, L'Aquila, Italy.

Endocrine Therapy

OT2-02-01 The SOLE Trial: International Breast Cancer Study Group (IBCSG 35-07) and Breast International Group (BIG 1-07) Study of Letrozole Extension
Colleoni M. SOLE Collaborative Group and International Breast Cancer Study Group.

OT2-03-01 Incidence of Mastalgia as a Presenting Complaint in Iranian Population with Regard to Age, BMI, Education, Residency (City or Rural), State of Marriage and Compare with Western Countries
Razavi S. Private Breast Clinic, Esfahan, Islamic Republic of Iran.

OT2-03-02 Prospective Neo-Adjuvant Registry Trial Linking MammaPrint, Subtyping and Treatment Response: Neoadjuvant Breast Registry - Symphony Trial (NBRST)
Whitworth P, Betsch P, Gentleman M, Akbari S. Nashville Breast Center, Nashville, TN; Dallas Surgical Group, Dallas, TX; Breast Care Specialists, Allentown, PA; Virginia Hospital Center, Arlington, VA.

OT2-03-03 Spectroscopic Feature of Breast Cancer

OT2-03-04 A Trial Model for the Future in the Search for Personalised Medicine - The UK POETIC and EPHOS-B Perioperative Trials Experience
Bliss JM, Robison LE, Webster-Smith MF, Emson MA, Kilburn LS, Smith IE, Robertson J, Dowsett M, Bundred NJ, Cameron DA, Vidya H, Horgan K, Evans AA, Kokan JS, Pinhel I, A'Hern R, on Behalf of the POETIC & EPHOS-B Trialists. Institute of Cancer Research, Sutton, London, United Kingdom; Royal Marsden Hospital, London, United Kingdom; Nottingham University Hospitals, Nottingham, United Kingdom; Royal Marsden Hospital and Breakthrough Breast Centre, London, United Kingdom; Wythenshawe Hospital, Manchester, United Kingdom; Western General Hospital, Edinburgh, United Kingdom; Stafford Hospital, Stafford, United Kingdom; Leeds General Infirmary, Leeds, United Kingdom; Poole Hospital, Poole, United Kingdom; Macclesfield District General Hospital, Macclesfield, United Kingdom.

OT2-03-05 Evaluation of the Prevalence and Prognostic Significance of VEGF 165s in Breast Cancer Patients Compared to Healthy Women

Chemotherapy

OT2-01-01 International Breast Cancer Study Group (IBCSG) Trial 22-00: Low-Dose Cytotoxics as Maintenance “Anti-Angiogenesis Treatment” Following Adjuvant Induction Chemotherapy for Patients with ER-Negative and PgR-Negative Breast Cancer
Colleoni M. International Breast Cancer Study Group.

OT2-01-02 First-Line Bevacizumab in Combination with Capecitabine or Paclitaxel for HER2-Negative Locally Recurrent or Metastatic Breast Cancer (LR/MBC): A Randomized Phase III Trial
Besl,ia Brodowicz, Greil, Inbar MJ, Kahain, Kaufman, Lang, Steger, Stemmer, Zielinski, Zierbale, The CECOG TURANDOT Trialists: Institute of Oncology, Sarajevo, Bosnia and Herzegovina; Medical University of Vienna, Vienna, Austria; University Hospital Salzburg, Salzburg, Austria; Tel Aviv Sourasky Medical Centre, Tel Aviv, Israel; University of Szeged, Szeged, Hungary; Sheba Medical Center, Tel Hashomer, Israel; National Institute of Oncology, Budapest, Hungary; Rabin Medical Center, Petah Tikva, Israel; Riga Eastern Clinical University Hospital, Riga, Latvia.
OT2-03-06  ACRIN 6698 MR Imaging Biomarkers for Assessment of Breast Cancer Response to Neoadjuvant Chemotherapy: A Sub-Study of the I-SPY 2 TRIAL (Investigation of Serial Studies To Predict Your Therapeutic Response with Imaging And molEcular Analysis)

Hylton NM, Partridge SC, Rosen M, Kim E, L’Heureux DZ, Esserman L, University of California at San Francisco, San Francisco, CA; University of Washington, Seattle, WA; Hospital of the University of Pennsylvania, Philadelphia, PA; Brown University, Providence, RI; American College of Radiology Imaging Network, Philadelphia, PA.

OT2-03-07  Withdrawn

Prevention

OT2-04-01  A Randomized Phase II Trial of Vitamin D vs Placebo in Premenopausal Women: CALGB 70806

Wood MC, Kingsley F, Ambaye AB, Yee L, Jung S-H, Marshall JR, Paskett E, University of Vermont, Burlington, VT; Ohio State University, Roswell Park Cancer Institute; Duke University.

OT2-04-02  Metformin for Breast Cancer Prevention: A Pilot Study

Ledgerwood NM, Djedja-Fourmer H, Patterson R, Hasteil F, Andre MP, Cadmus L, Blair S, University of California, San Diego, CA.

OT2-04-03  Uptake of a Randomized Breast Cancer Prevention Trial Comparing Letrozole to Placebo in BRC1/2 Mutations Carriers: The FNCLCC ONCO-03/LIBER Trial

Pujal P, Lasset C, Berthet P, Dugast C, Delagelle S, Fricker JP, Chabbert Buffet N, Lemonnier J, Rocca L, Mjolmen S, Baudry K, Martin AL, University Hospital CHU Amiens de Villeneuve, Montpellier, France; Centre Léon Bérard, Lyon and Université de Lyon 1; CNRS; UMR 5558, Villeurbanne, France; Centre Francois Baclesse, Caen, France; Institut Gustave Roussy, Villejuif, Centre Paul Strauss, Strasbourg, AP HP Hôpital Tenon; Fédération Nationale Française des Centres de Lutte Contre le Cancer.

Imaging

OT2-05-01  An Open-Label Positron Emission Tomography Study To Investigate and Quantify Brain and Tumor Penetration of Carbon-11-Labeled Lapatinib in Patients with HER2-Overexpressing Advanced or Metastatic Breast Cancer


OT2-05-02  ACRIN 6691 Monitoring and Predicting Breast Cancer Neoadjuvant Chemotherapy Response Using Diffuse Optical Spectroscopic Imaging (DOSI)

Trombregh BJ, L’Heureux DZ, Mankoff DA, Zhang Z, Cerussi A, Mehta R, Carpenter PM, Butler JA, Hylton NM, Kaufman P, Pogue BW, Paulsen K, Yodh AG, Boas DA, Isakoff S, University of California, Irvine, CA; American College of Radiology Imaging Network, Philadelphia, PA; University of Washington, Seattle, WA; Brown University, Providence, RI; University of California at San Francisco, San Francisco, CA; Dartmouth University, Lebanon, NH; University of Pennsylvania, Philadelphia, PA; Massachusetts General Hospital, Charlestown, MA; Massachusetts General Hospital, Boston, MA.

OT2-05-03  ACRIN 6688 Phase II Study of Fluorine-18 3'-Deoxy-3'-Fluorothymidine (FLT) in Invasive Breast Cancer

Jollès P, Kontakouglu L, Bear HE, Iwado MO, Kurzziel K, Shankar L, Mankoff DA, Duan F, L’Heureux DZ, Virginia Commonwealth University, Richmond, VA; Mount Sinai School of Medicine, New York, NY; National Cancer Institute, Bethesda, MD; University of Washington, Seattle, WA; Brown University, Providence, RI; American College of Radiology Imaging Network, Philadelphia, PA.

OT2-05-04  ACRIN PA 4006: Comparison of Full-Field Digital Mammography with Digital Breast Tomosynthesis Image Acquisition in Relation to Screening Call-Back Rate

Conant EF, Maidment A, Copt D, Olson CB, Heckel ML, Gatsonis C, Hospital of the University of Pennsylvania, Philadelphia, PA; Albert Einstein Medical Center, Philadelphia, PA; American College of Radiology Imaging Network, Philadelphia, PA; Brown University, Providence, RI.

OT2-05-05  Phase II/II Study of Adoptive T Cell Therapy Following In Vivo Priming with a HER2 Peptide-Based Vaccine in Patients with Stage IV Breast Cancer

Parker SL, Higgins DM, Childs JS, Dang Y, Guthrie KA, Disis ML, Salazar LG, Covelar A, University of Washington, Seattle, WA; Fred Hutchinson Cancer Research Center, Seattle, WA.

OT2-05-06  ACOSOG Z11101/ACRIN 6694: Effect of Preoperative Breast MRI on Surgical Outcomes, Costs and Quality of Life of Women with Breast Cancer

Bedrossian J, Suman VJ, Yao K, Shih Y-CT, Yen TWF, Comstock C, Newsstead G, Birdwell R, Kim E, L’Heureux DZ, Gatsonis C, MD Anderson Cancer Center, Houston, TX; Mayo Clinic, Rochester, MN; University of Chicago, Evanston, IL; University of Chicago, Chicago, IL; Medical College of Wisconsin, Milwaukee, WI; Memorial Sloan-Kettering Cancer Center, New York City, NY; Harvard Medical School, Boston, MA; Brown University, Providence, RI; American College of Radiology Imaging Network, Philadelphia, PA.

Radiotherapy

OT2-06-01  A Phase III Randomized Multicentric French Study To Evaluate the Impact of a Localized 16-Gy Boost after Conservative Surgery and a 50-Gy Whole-Breast Irradiation in Breast Ductal Carcinoma In Situ (The BONBIS Trial)

Azria O, Cowen D, De La Lande B, Bourger C, Latorzeff I, Leblanc-Ofroy M, Douadi-Gaci Z, Pradier O, Peignaux K, Levy C, Ellis S, Lecouillard I, Racadot S, Bontemps P, Berinyock P, La Garde N, Laharie-Mineur H, Cretin J, Marchal C, Serin D, Lemanski C, CRLC Val d’Aurelle, Montpellier, France; AP-HM, Marseille, France; Centre Rene Huguenin, Saint Cloud, France; IGR, Villejuif, France; Groupe ONCOCRAD, Toulouse, France; Centre Rene Gauducheau, Nantes, France; Centre Catherine de Sienne, Nantes, France; CHU Brest, Brest, France; CRLC G-F, Clermont, Tours, France; CRLC Francois Baclesse, Caen, France; Centre Catalan d’Oncologie, Perpignan, France; CRLC Eugene Marquis, Rennes, France; Centre Leon Berard, Lyon, France; CHU Besancon, Besancon, France; Centre Henri Becquerel, Rouen, France; Institut Bergonie, Bordeaux, France; Clinique Tivoli, Bordeaux, France; Clinique Valdegouer, Nimes, France; Centre Alexis Vautrin, Vandoeuvre les Nancy, France; Institut Sainte Catherine, Avignon, France.

OT2-06-02  A Randomized Phase III Study of Conventional Whole Breast Irradiation (WBI) vs Partial Breast Irradiation (PBI) for Women with Stage 0, 1, or 2 Breast Cancer: NSABP B-39/RTOG 0413

Julian TB, Costantino JP, Vicini FA, White JR, Cecchini RS, Winter KA, Arthur DW, Kuske R, Rabinovitch R, Parida DS, Mamouns EP, Curran Jr WJ, Wolmark N, National Surgical Adjuvant Breast & Bowel Project (NSABP), Pittsburgh, PA; Allegheny General Hospital, Pittsburgh, PA; NSABP Biostatistical Center, Pittsburgh, PA; University of Pittsburgh Graduate School of Public Health, Pittsburgh, PA; Beaumont Health System, Royal Oak, MI; Radiation Therapy Oncology Group (RTOG), Philadelphia, PA; Medical College of Wisconsin, Milwaukee, WI; Virginia Commonwealth University, Richmond, VA; Arizona Breast Cancer Specialists, Scottsdale, AZ; University of Colorado Denver, Aurora, CO; Aultman Health Foundation, Canton, OH; Emory University, Atlanta, GA.
Side-effect Control

OT2-07-01 Pasireotide Long Acting Release (LAR) in Breast Cancer Patients To Prevent Lymphocytosis after Mastectomy and Axillary Node Dissection: A Randomized, Multicenter, Phase II Study

OT2-07-02 SWOG S0927: A Randomized Double Blind Placebo-Controlled Trial of -3-Fatty Acid for the Control of Aromatase Inhibitor (AI)-Induced Musculoskeletal Pain in Women with Early Stage Breast Cancer
Hershman DL, Unger JM, Crow KD, Monpourn CR, Minasian LM, Hansen L, Lew DL, Kabelke K, Wade JL, Meyskens FL. Columbia University, New York, NY; SWOG, Seattle, WA; National Cancer Institute, Bethesda, MD; Legacy Good Samaritan Hosp & MC, Portland, OR; SWOG, San Antonio, TX; Cancer Care Specialists of Illinois, Decatur, IL; University of California, Irvine, Orange County, CA.

7:30 pm–9:30 pm
OPEN SATellite EVENT
Marriott Rivercenter, Grand Ballroom
Targeting breast Cancer Molecular Phenotypes: A Dialogue Between Community and Academic Oncologists
WEBSITE: http://primeoncology.org/sanantoniosymposium2011

FRIDAY, DECEMBER 9, 2011

6:45 am–5:15 pm
REGISTRATION
Bridge Hall

7:00 am–9:00 am
POSTER SESSION 4 & CONTINENTAL BREAKFAST
Exhibit Halls A-B

Tumor Cell Biology: Endocrine Therapy and Resistance

P4-01-01 Preclinical and Clinical Studies of Estrogen Deprivation Support the PDGF/Abl Pathway as a Novel Therapeutic Target for Overcoming Resistance

P4-01-02 Endocrine Resistance: Mechanism, Tumorogenic Capacities, and New Therapeutic Strategies
Morrison GD, Fu X, thimakin S, rimawi MF, wicha MS, Osborne CK, Schirf R. Baylor College of Medicine, Houston, TX; University of Michigan, Ann Arbor, MI.

P4-01-03 Establishment and Characterization of an Endocrine Resistance Model In Vitro and In Vivo by Inducible PTEN Knockdown
Fu X, Shea M, Biswal NC, Mitchell T, Giuliano M, Healy NA, Meerbrey KL, Joshi A, Westbrook T, Hilsenbeck SG, Osborne CK, Schirf R. Baylor College of Medicine, Houston, TX; National University of Ireland, Galway, Ireland.

P4-01-04 Effects of CYP2D6 Phenotype and Drug Adherence on Tamoxifen Metabolite Levels

P4-01-05 The Multikinase Inhibitor Sorafenib Can Overcome Antiestrogen Resistance in Patients with Progressive Metastatic Estrogen Receptor (ER) Positive Breast Cancer

P4-01-06 Global Characterization of the SRC-1 Transcriptome Identifies Disintegrin C as an ER-Independent Mediator of Endocrine Resistant Breast Cancer
Bolger JC, McCartan DP, McIlroy M, Byrne C, Fagan A, Xu J, O’Gara P, Hill ADK, Young LS. Royal College of Surgeons in Ireland, Dublin, Ireland; University College Dublin, Dublin, Ireland; Dan L. Duncan Cancer Center, Baylor College of Medicine, Houston, TX.

P4-01-07 Combining Hedgehog Inhibitor, GDC-0449 with Tamoxifen Overcomes Tamoxifen-Resistance in In-Vitro and In-Vivo Studies of Estrogen-Receptor Positive Breast Cancer
Majumder S, Lu Y, Teng K-y, Kaffenberger T, Zhang X, Nuovo G, love RR, Ramsawamy B. The Ohio State University, Columbus, OH; International Breast Cancer Research Foundation, Madison, WI.

P4-01-08 The Role of Ser991 PELP1 Phosphorylation in Therapy Resistance and Metastasis of Breast Cancer
Sareddy GR, Nair BC, Krishnan SR, Konugunta VK, Vakalambudi RK. UTHSCSA, San Antonio, TX.

P4-01-09 Reduced CYBA Expression Leads to the Development of Fulvestrant Resistance in a Breast Cancer Cell Line
Skerri BJO, Ramsahoye BH. The University of Edinburgh, Edinburgh, United Kingdom.

P4-01-10 The Role of the Steroid Receptor Coactivator SRC1 and Its Functional Partner HOXC11 in the Development of Endocrine Resistant Breast Cancer
Walsh CA, McCartan D, Hill ADK, Young LS. Royal College of Surgeons in Ireland, Dublin, Ireland.

P4-01-11 Autocrine Human Growth Hormone (hgh) Reduces the Sensitivity of Breast Cancer Cells to Treatment with Tamoxifen and Fulvestrant
Perry JK, Chen H, Bougen NM, Liu D-X, Lobie PE. University of Auckland, Auckland, New Zealand; National University of Singapore, Singapore.

P4-01-12 The Unfolded Protein Response (UPR) and Pro-Survival Autophagy Contributes to Antiestrogen Resistance in Breast Cancer
Clarke R, shajahan AN, Cook KL, Hickman FE, Facey CO. Georgetown University, Lombardi Comprehensive Cancer Center, Washington, DC.

P4-01-13 Biology of Aggressiveness and Tamoxifen Resistance in Hormone Receptor-Positive Very Young Age Breast Cancer
Han W, Ro J, Jung S. Seoul National University Hospital, Seoul, Korea; National Cancer Center, Goyang, Kyunggi, Korea.

P4-01-14 Fulvestrant Regulates Epidermal Growth Factor (EGF) Ligands and Induces EGF Receptor Activation in MCF-7 Breast Cancer Cells
Zhang X, Diaz MR, Yee D. University of Minnesota, Minneapolis, MN.

P4-01-15 Alteration of Y-box Binding Protein-1 Expression Modifies the Response to Endocrine Therapy in Estrogen Receptor Positive Breast Cancer
Ikeda T, Kamijo S, Isumi H, Kohno K, Ito K-i. shirushi University School of Medicine, Matsumoto, Nagano, Japan; University of Occupational & Environmental Health, Kitakyo, Fukuoka, Japan.

P4-01-16 The Influence of CYP2D6 Genetic Polymorphisms on Variability of Tamoxifen Metabolism in the Lebanese Breast Cancer Population
P4-01-17  TIMP-1 Over-Expression Confers Resistance of MCF-7 Breast Cancer Cells to Fulvestrant
Bjerre CA, Vinther L, Belling K, Schroih Rasmussen A-S, Li J, Lin X, Han Z, Wang J, Bolund L, Jensen V, Nielsen BS, Soekilde R, Gupta R, Lademann U, Brunner N, Stenvang J. Faculty of Life Sciences, University of Copenhagen, Copenhagen, Denmark; Technical University of Denmark, Lyngby, Denmark; Aarhus University, Aarhus, Denmark; BGI-Shenzhen, Shenzhen, China; Eotop AS, Vedbeak, Denmark.

P4-01-18  AP-1 Blockade Potentiates the Anti-Tumor Effect of Endocrine Treatment and Reverts the Resistant Phenotype in Hormone Receptor-Positive Breast Cancer
Malorni L, Giuliano M, Migliaccio I, Wang T, Creighton CJ, Lupien M, Hilsenbeck SG, Healy N, Mazumdar A, Trivedi MV, Jeselsohn R, He HH, Fu X, Gutierrez C, Brown M, Brown PH, Osborne CK, Schiff R. Baylor College of Medicine, Houston, TX; Hospital of Prato, Prato, Italy; The University of Texas M.D. Anderson Cancer Center, Houston, TX; Dana-Farber Cancer Institute and Harvard Medical School, Boston, MA; Dana-Farber Cancer Institute, Boston, MA; Beth Israel Deaconess Medical Center, Boston, MA; Dartmouth Medical School, Lebanon, NH; UH College of Pharmacy, Houston, TX.

P4-01-19  SRC-1 Expression Is a Prognostic Factor of Breast Cancer-Specific and Disease-Free Survival in Inflammatory Breast Cancer

P4-01-20  Specific Anti-Proliferative Profile of Endoxifen for MCF-7 Cell Compared to 4-OH Tamoxifen
Saji S, Hirose M, Hayashi S-i, Kuroi K. Saitama Medical University, IMC, Saitama, Japan; Tokyo Metropolitan Komagome Hospital, Tokyo, Japan; Tohoku University Graduate School of Medicine, Sendai, Japan; Kyoto University, Graduate School of Medicine, Kyoto, Japan.

P4-01-21  An Estrogen-Inducible Transcription Factor FOXP1 Promotes Estrogen-Dependent Cell Proliferation of Breast Cancer Cells and Is Associated with 5-Year Disease-Free Survival in Patients with Tamoxifen-Treated Breast Cancer
Shigekawa T, Ichij N, Ikeda K, Hone-Inoue K, Shimizu C, Saji S, Aogi K, Tsuchida H, Osaki A, Sasaki J, Inoue S. Tokyo Metropolitan Cancer and Infectious Disease Center, Komagome Hospital; Research Center for Genomic Medicine, Saitama Medical University; International Medical Center, Saitama Medical University; National Cancer Center Hospital; National Shikoku Cancer Center; Graduate School of Medicine, The University of Tokyo.

P4-01-22  Cyclin D1 and Its Prognostic Value in Planning of Endocrine Therapy for Women of Postmenopausal Age with Breast Cancer
Skvortsov V, Manikhas A, Manikhas G, Oganessian A, Raskin G. City Cancer Therapy for Women of postmenopausal age with breast Cancer and is associated with 5-year Disease-Free Survival in patients with tamoxifen-treated breast Cancer; Faculty of Life Sciences, University, Columbus, OH.

Tumor Cell Biology: Hormonal Factors and Receptors

P4-02-01  Bis(2-Ethylhexyl) Phthalate: A Potential Endocrine Disruptor Induces Letrozole Insensitivity and Induction of Aromatase Activity in Breast Cancer Cells
Nair HB, Bhaskaran SS, Krishnegowda NK, Tekmal RR, Vandenberg JL. Texas Biomedical Research Institute, San Antonio, TX; University of Texas Health Science Center, San Antonio, TX.

P4-02-02  The Natural Fetal Estrogen Estetrol (E4), Causes Anti-Estrogenic Effects in Women with Endocrine-Responsive Positive Early Breast Cancer
Singer CF, Natter C, Steurer S, Radas M, Moinfar F, Appels NRCA, Visser M, Kubista E, Bennink C. Medical University of Vienna, Wien; Medical University of Graz, Wien; Pantaerheie Bioscience.

Tumor Cell Biology: Animal Models

P4-03-01 Stat5 regulates the PI3-Kinase/Akt1 pathway during Mammary Gland Development and Promotes Neoplastic Transformation in a Mouse Model for Cowden Syndrome
Schmidt JW, Sakamoto K, Creamer BA, Levine G, Wagner K-U. University of Nebraska Medical Center, Omaha, NE; Ohio State University, Columbus, OH.

P4-03-02  Biological Functions of Estrogen Receptor-beta and Its Variants in Breast Cancer

P4-03-04  Androgen Receptor (AR) Expression in a Cohort of Patients (pts) with Triple Negative Breast Cancer (TNBC)

P4-03-05  The Regulation of Artemin Signalling by IGF-1 in Mammary Carcinoma Cells
Yip C, Liu D, Lobie PE, Perry JK. University of Auckland, Auckland, New Zealand; National University of Singapore, Singapore.

P4-03-06  Progesterone Receptor Expression Predicts Poor Outcome in Estrogen Receptor Positive, Lymph Node Negative Breast Cancer - A Population Based Study
Purdie CA, Quinlan P, Jordan LB, Ashfield A, Baker L, Dewar JA, Thompson AM. Ninewells Hospital & Medical School, Dundee, United Kingdom; University of Dundee, Dundee, United Kingdom.

P4-03-07  Influence of the Progesterone Receptor on the Prognosis of Breast Cancer in Interaction with Other Prognostic Factors

P4-03-08  Obesity-Induced Aromatase Expression in the Breast Microenvironment Promotes Estrogen Receptor Activity and Circulating Estradiol Levels
Bowers LW, Brenner AJ, Li R, Tekmal RR, deGraffenried LA. The University of Texas at Austin, Austin, TX; The University of Texas Health Science Center at San Antonio, San Antonio, TX.

P4-03-09  Estrogen-Related Receptor a mediates Insulin-Like Growth Factor-I Dependent Migration in Breast Cancer Cells
Fettig-Anderson LM, Oh AS, Yee D. University of Minnesota, Minneapolis, MN.

P4-03-10  The Relationship between Estrogen Receptor Gene Polymorphism and Mammographic Density in Postmenopausal Women
Baldisserotto FDG, Elias S, Silva IDCG, Nazario ACP. Universidade Federal de Sao Paulo - UNIFESP-EPMS, Sao Paulo, SP, Brazil.

P4-03-11  Insulin-Like Growth Factor I Promotes Estrogen Receptor Positive Breast Cancer Cell Proliferation, in Part, through CYPIA1 Signaling
Rodriguez M, Becker M, Yee D, Potter D. University of Minnesota, Minneapolis, MN.

P4-03-12  Plasma Estradiol Levels and Degree of Estrogen Receptor Positivity by Image Analysis in a Large Cohort of Breast Cancer Cases: Results from the Nurses’ Health Study
Collins LC, Frielin GW, Ahern TP, Hu R, Hankinson SE, Tamimi RM. Beth Israel Deaconess Medical Center, Boston; Harvard Medical School, Boston; Channing Laboratory, Brigham and Women’s Hospital, Boston; Harvard School of Public Health, Boston.

Cancer Res; 71(24 Suppl.) December 15, 2011
52s Cancer Research
P4-03-02  High Fat Diet-Induced Postmenopausal Obesity Promotes Tumor Angiogenesis and Breast Cancer Progression in Age-Relevant Ovariectomized Mice  
Gu J-W, Young E, Patterson SG, Makey KL, Huang M, Tucker KB, Chinch E, Miele L. University of Mississippi Medical Center, Jackson, MS.

P4-03-03  Therapeutic Sensitivities of Mouse Models of Human Breast Cancer  

P4-03-04  Identification of Molecular Targets for Cancer-Initiating Cells Using a Triple-Negative Breast Cancer Mouse Model  
Kai I, Iwamoto T, Pusztai L, Hortobagyi GN, Saya H, Ueno NT. The University of Texas MD Anderson Cancer Center, Houston, TX; Institute for Advanced Medical Research, Shinjuku-ku, Tokyo, Japan.

P4-03-05  Development of an Inducible Estrogen Receptor Co-Activator PELP1 Mammary Tumor Model  
Cortez VA, Newaldo D, Chodosh LA, Tekmal RR, Vadlamudi RK. The University of Texas Health Science Center at San Antonio, San Antonio, TX; University of Pennsylvania School of Medicine, Pennsylvania, PA.

P4-03-06  Development and Comparative Characterization of Metastasis in Newly Developed Pre-Clinical Models of Inflammatory Breast Cancer  
Chu K, Mu Z, Alpaugh KR, Fernandez S, Freiter EM, Wu H, Zook MB, Barsky SH, Cristofanilli M, Robertson FM. The University of Texas MD Anderson Cancer Center, Houston, TX; Fox Chase Cancer Center, Philadelphia, PA; University of Nevada School of Medicine, Reno, NV; Nevada Cancer Institute, Las Vegas, NV.

Tumor Cell Biology: Mammary Development and Differentiation

P4-04-01  Identification of a Unique Mammary Cell Type Expressing Mesenchymal Markers, but Capable of Multilineage Epithelial Differentiation  
Landua JD, Lewis MT. Baylor College of Medicine, Houston, TX.

P4-04-02  Smoothened Function as a G-Protein Coupled Receptor in Mammary Epithelial Cells  
Villanueva H, Vishal AP, Bimbauer L, Plummer NW, Lewis MT. Baylor College of Medicine, Houston, TX; National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Tumor Cell Biology: Metabolism and Breast Cancer

P4-05-01  Rab25 Alters Cellular Energetics and Growth Signaling during Breast Oncogenesis  
Mitra S, Cheng KW, Dennison JB, Mills GB. MD Anderson Cancer Center, Houston, TX.

P4-05-02  Early Developmental Exposures to a High Carbohydrate/High Fat Diet Affect Glucose Metabolism and Mammary Cancer Susceptibility  
Berton TR, Lambertz I, Tian J, Johanning G, Conti C, Fuchs-Young R. UT MD Anderson Cancer Center, Smithville, TX; UT MD Anderson Cancer Center, Bastroop, TX.

P4-05-03  Mechanism Underlying Metabolic Heterogeneity in Breast Cancer Subtypes  
Molina JR, Dennison JB, Zhang F, Mills GB. MD Anderson Cancer Center, Houston, TX.

P4-05-04  Arachidonic Acid-Induced Elevated Expression of 5-Lipoxygenase Is Linked to Metastatic Migration of Breast Cancer Cells  

P4-05-05  Imaging Mass Spectrometry Based Lipid Metabolites Analysis for Breast Cancer  
Ide Y, Nishio T, Hosokawa Y, Matsunuma R, Koizumi K, Ogura H, Shiya N, Setou M. Hamamatsu University School of Medicine, Hamamatsu, Japan.

P4-05-06  Systemic Cholesterol promotes Breast Tumor Aggressiveness  
Santos CR, Almeida JM, Dias S. Portuguese Institute of Oncology, Lisbon, Lisbon, Portugal.

P4-05-07  Insulin-like growth factor 1 induces a rewiring of the aerobic glycolysis pathway in breast cancer  
Pinto DM, Murphy JP, Chisholm KA, Drucker D. National Research Council of Canada, Halifax, Nova Scotia, Canada; Harvard University, Boston, Massachusetts, United States; Queen Elizabeth II Health Sciences Centre, Halifax, Nova Scotia, Canada.

P4-05-08  Metabolomic Profiling of Breast Cancer Delineates Clinical and Race-Specific Subtypes and Associated Bioprocesses  
Putluri N, Dorsey T, Golffmann L, Nanda S, Putluri V, Wallace T, Mathi E, Zhang X, Tenunuma A, Michalidis G, Lewis M, Schirr R, Amb S, Seekumar A. Baylor College of Medicine, Houston, TX; National Cancer Institute, Bethesda, Maryland; University of Michigan, Ann Arbor, MI.

Tumor Cell Biology: Micrometastases

P4-06-01  Withdrawn

P4-06-02  Microscopic Disease in Blood and Bone Marrow Predicts Survival in Early Stage Breast Cancer  
Lucci A, Krishnamurthy S, Lodhi A, Bhattacharyya A, Hall C, Anderson A, Beddosian I, Singh B, Kuerer H. University of Texas MD Anderson Cancer Center, Houston, TX.

P4-06-03  Multiplex Gene Expression of Disseminated Tumor Cells in the Bone Marrow of Breast Cancer Patients Identifies Novel Therapeutic Targets  
Aft R, Mudalagiriyappa C, Pillai S, Fleming T, Watson M. Washington University, St. Louis, MO.

P4-06-04  Detection of HER2 Gene Amplification in Circulating Tumor Cells and Disseminated Tumor Cells by Fluorescence In Situ Hybridization Using OncoCee™  
Krishnamurthy S, Bischoff FZ, Mayer JA, Wong K, Pham T, Kuerer HM, Lodhi AK, Bhattacharyya A, Hall C, Lucci A. University of Texas M.D. Anderson Cancer Center, Houston, TX; Biocept Laboratories, San Diego, CA.

P4-06-05  Prognostic Impact of Disseminated and Circulating Tumor Cells in Patients Treated for Locally Advanced Breast Cancer  
Mathiesen RR, Neslund JM, Renolten A, Lakkevik E, Anjer G, Ostenstad B, Lundgren S, Riiberberg T, Mjaaland I, Ovalheim G, Lønning PE, Naume B. Oslo University Hospital The Radium Hospital, Oslo, Norway; Oslo University Hospital, Oslo, Norway; University of Bergen, Bergen, Norway; Haukeland University Hospital, Bergen, Norway; St. Olavs University Hospital, Trondheim, Norway; Norwegian University of Science and Technology, Trondheim, Norway; University Hospital of Northern Norway and Institute of Clinical Medicine, University of Tromsø, Tromsø, Norway; Stavanger University Hospital, Stavanger, Norway; Oslo University Hospital, The Radium Hospital, Oslo, Norway; University of Oslo, Oslo, Norway.

P4-06-06  Morphological Categories of ICC-Detected CK+ Cells in Bone Marrow Have Different Prognostic Impact in Breast Cancer  
Borgen E, Symnestsved M, Schirmer CB, Schlichting E, Nesland JM, Naume B. The Radium Hospital, Oslo University Hospital, Oslo, Norway; The Radium Hospital, Oslo University Hospital, Oslo, Norway; Ullevål Hospital, Oslo University Hospital, Oslo, Norway; University of Oslo, Oslo, Norway.

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Detection and Diagnosis – Pathology: Circulating Tumor Cells

Circulating Tumor Cells, Disease Recurrence and Survival in Newly Diagnosed Breast Cancer
Franken B, de Groot MR, Tenstappen LWWM, Mastboom WJB, Van der Palen J, Tibbe AGJ. Medisch Spectrum Twente, Enschede, Overijssel, Netherlands; University of Twente, Enschede, Overijssel, Netherlands.

Detection, Enrichment, Characterization and Propagation of Circulating Tumor Cells from Patients with Advanced Metastatic Breast Cancer
Mihalicou C, Lian J, Bertos N, Omeroglu A, Sebag M, DiBattista J, Li J, Chughtai N, Park M, Kremer R, Royal Victoria Hospital/McGill University, Montreal, QC, Canada; McGill University, Montreal, QC, Canada.

Identification of Triple-Negative Primary Breast Cancer Xenograft Models with High Numbers of Circulating and Disseminated Tumor Cells
Giuliano M, Christy PJ, Zhang X, Mao S, Contreras A, Lewis MT, Rimawi MF, Osborne CK, Schiff R, Trivedi MV. Baylor College of Medicine, Houston, TX; UH College of Pharmacy, Houston, TX.

Nomogram Including Circulating Tumor Cells (CTC) Count before and during Chemotherapy for Individual Survival Prediction of Metastatic Breast Cancer Patients
Bidard F-C, Hajage D, Bachelot T, Delaloge S, Brain E, Campone M, Wolp-Diniz R, Dieras V, Mathiot C, Asselain B, Pierga J-Y. Institut Curie, Paris, France; Centre Leon Berard, Lyon, France; Institut Gustave Roussy, Villejuif, France; Institut de Cancérologie de l’Ouest, Nantes, France.

Comparison of PIK3CA Hot Spot Mutations in the Primary Tumor or Metastases with PIK3CA Mutations or PIK3CA Over-Expression in Circulating Tumor Cells of Metastatic Breast Cancer Patients under Sequential Palliative Therapy
Aktas B, Kasimir-Bauer S, Kasper S, Derks C, Kimmig R, Schuler M, Tewes M. West German Cancer Center, University Hospital Essen, Germany; University of Duisburg-Essen, Essen, Germany.

Correlation of Two Analytical Methods for Circulating Tumor Cells in Peripheral Blood of Patients with Primary Breast Cancer
Jaeger BAS, Rack B, Jueckstock J, Salmen J, Oettmann U, Lorenz R, Rezaiz M, Beck T, Schneeweß A, Zwingers T, Beckmann MW, Friese K, Janni W. Klinikum der Ludwig-Maximilians-Universität - Campus Innenstadt, Munich, Germany; Heinrich Heine-Universität, Duesseldorf, Germany; Gemeinschaftspraxis Lorenz-Hecker-Wesche, Braunschweig, Germany; Lusenkrankenhaus, Duesseldorf, Germany; RoMed Klinikum Rosenheim, Rosenheim, Germany; University Hospital Heidelberg, Heidelberg, Germany; Estimate, Augsburg, Germany; Frauenklinik der Universitaet Erlangen, Erlangen, Germany.

Circulating Tumor Cells Predict Survival in Non-Metastatic Breast Cancer
Lucci A, Krishnamurthy S, Bhattacharyya A, Lodhi A, Hall C, Singh B, Anderson A, Bedrosian I, Kuerer H. University of Texas MD Anderson Cancer Center, Houston, TX.

Subsets and Molecular Signatures of Circulating Tumor Cells in Breast Cancer Brain Metastasis
Marchetti D, Zhang L, Wetzel M, Zaidi T, Ridgway M, Schoeber W, He W, Groves MD, Katz RL. Baylor College of Medicine, Houston, TX; MD Anderson Cancer Center, Houston, TX.

Automated Quantitative Assessment of HER2 Expression of Circulating Tumor Cells (CTC) in Metastatic Breast Cancer (IC 2006-04 Study)
Bidard F-C, Ligthart ST, Decraene C, Bachelot T, Delaloge S, Brain E, Campone M, Pierga J-Y, Tenstappen LWWM. Institut Curie, Paris, France; University of Twente, Enschede, Netherlands; Centre Leon Berard, Lyon, France; Institut Gustave Roussy, Villejuif, France; Institut de Cancérologie de l’Ouest, Nantes, France.

Circulating Tumor Cells in Newly Diagnosed Inflammatory Breast Cancer
Mego M, Giordano A, De Giorgi U, Hsu L,ucci A, Dawood S, Woodward WA, Ueno NT, Valero V, Andreoupolou E, Hortobagyi GN, Reuben JM, Cristofanilli M. Comenius University, School of Medicine, Bratislava, Slovakia (Slovak Republic); UT MD Anderson Cancer Center, Houston, TX; Fox Chase Cancer Center, Philadelphia.

Circulating Tumor Cells after Neoadjuvant Therapy Predict Outcome in Stage I to Ill Breast Cancer
Gainer S, Krishnamurthy S, Bhattacharyya A, Lodhi A, Hall C, Kuerer H, Bedrosian I, Anderson A, Singh B,ucci A. The University of Texas MD Anderson Cancer Center, Houston, TX.

Identification of p53 Mutation in Whole Genome DNA from Single Circulating Tumor Cells (CTCs) and Primary Breast Cancers (BC) from Patients (pts) with Metastatic Breast Cancer (MBC)

Prognostic Impact of Circulating Tumor Cells Assessed with the Cell Search Assay and Adna Test Breast in Metastatic Breast Cancer Patients - The DETECT Study
Mueller V, Riehensperger S, Rack B, Wolfgang J, Paschinger PA, Solomayer E, Aktas B, Kasimir-Bauer S, Mury D, Pental K, Fehrm T. University Medical Center Hamburg-Eppendorf, LMU Munich; University Medical Center Dusseldorf; Hospital Erlangen, Friedrich-Alexander University Erlangen Nuremberg, Erlangen; University Medical Center Hamburg-Saar; University Medical Center Essen; University Medical Center Tubingen, All Authors on Behalf of the DETECT Study Group.

Circulating Tumor Cells (CTCs) Detection and HER2 Profiling by CellSearch® in Non-Metastatic Breast Cancer: An International Ring Study To Assess Inter-Reader Variability
Ignatiadis M, Pierga J-Y, Campion M, Fehrm T, Payne R, Rack B, Pavlidis D, Riehensperger S, Rothe F, Besi S, Auma C, Sandri MT, Borgen E, Kraan J, Tenstappen LWWM, Picart M, Sotiriou C, Michalis, S, Pantel K. Institut Jules Bordet, Brussels, Belgium; Institut Curie, Paris, France; Mayo Clinic, Rochester, Universitäts-Frauenklinik, Tubingen, Germany; Hamersmith Hospital, Imperial College, London, United Kingdom; Ludwig-Maximilians-Universitat, Munchen, Germany; University of Crete, Crete, Greece; University Medical Center Hamburg-Eppendorf, Hamburg, Germany; Hospital of Prato, Prato, Italy; Vall d’Hebron, Barcelona, Spain; European Institute of Oncology, Milan, Italy; Oslo University Hospital, Oslo, Norway; Erasmus Medical Center, Rotterdam, Netherlands; University of Twente, Twente, Netherlands.

Insulin-Like Growth Factor Receptor I (IGFIR) Expression in Circulating Tumor Cells (CTCs) of Patients with Early and Metastatic Breast Cancer
Spiliotis M, Markanomolakis H, Kokotsaki M, Kallergi G, Pavlidis D, Georgoulis V, Agelaki S. School of Medicine, University of Crete, Heraklion, Crete, Greece; University General Hospital of Heraklion, Heraklion, Crete, Greece.

Development of Circulating Tumor Cell-Endocrine Therapy Index in Metastatic Breast Cancer Patients

Isolation of Highly Pure Circulating Tumor Cells (CTCs) from Metastatic Breast Cancer (MBC) Patients for Gene Expression Analysis
P4-07-18  Prospective Assessment of Circulating Tumor Cells and Serum Markers for PFS Prediction in Metastatic Breast Cancer
Bidard F-C, Hajage D, Bachelot T, Delaloge S, Brain E, Campone M, Cottu P, Beuzeboc P, Mathiot C, Perga J-Y. Institut Curie, Paris, France; Centre Léon Bérard, Lyon, France; Institut Gustave Roussy, Villejuif, France; Centre René Gauduchau, Nantes, France.

P4-07-19  Bone Marrow Involvement is Associated with High Numbers of Circulating Tumor Cells in Peripheral Blood of Metastatic Breast Cancer Patients
Grisanti S, Consolfi F, Almici C, Bertagna F, Versardi R, Ungari M, Amoroso V, Pedersini V, Rassali I, Montani E, Simoncini EL. Spedale Civili di Brescia, Brescia, Italy; University of Brescia, Brescia, Italy.

P4-07-20  Apoptosis in Circulating Tumor Cells (CTCs) of Early and Metastatic Breast Cancer Patients
Kallergi G, Konstantinidis G, Papadaki M, Agelaki S, Movroudis D, Stournaras C, Georgoulas V. School of Medicine, University of Crete, Heraklion, Crete, Greece; University General Hospital of Heraklion, Heraklion, Crete, Greece.

P4-07-21  A Comparison of Two Methods for the Detection of Circulating Tumor Cells (CTCs) in Patients (pts) with Early and Metastatic Breast Cancer (BC): RT-PCR for Cytokeratin (CK) -19 mRNA Versus the CellSearch System
Politaki E, Agelaki S, Apostolaki S, Perraki M, Hatzidaki D, Lianidou ES, Movroudis D, Georgoulas V. University General Hospital of Heraklion, Heraklion, Crete, Greece; University of Athens, Athens, Greece.

Detection and Diagnosis – Pathology: Circulating Markers

P4-08-01  Predictive Value of HER2 Serum Levels in Patients Treated with Lapatinib or Trastuzumab - A Translational Project in the Neoadjuvant “Geparquinto” Trial
Witzel ID, Loibl S, von Minckwitz G, Abdallah A, Kühn T, Overkamp F, Fehm T, Schrader I, Ueler C, Kohls A, Strumborg S, zu Eulenburg C, Untch M, Müller V. University Medical Center Hamburg-Eppendorf, Hamburg, Germany; German Breast Group, Neu-Isernburg, Germany; Helios Klinikum Berlin Buch, Berlin, Germany; University Medical Center Tubingen, Tubingen, Germany; Hospital Gelsenkirchen, Gelsenkirchen, Germany; Hospital Esslingen, Esslingen, Germany; Oncologianova, Recklinghausen, Germany; Gynäko-Onkologische Praxis Hannover, Hannover, Germany; Gynäkologische Gemeinschaftspraxis, Hildesheim, Germany; Ev. Krankenhaus, Ludwigsfelde-Teltow, Germany; Frauenklinik Rheinfelden, Rheinfelden, Germany.

P4-08-02  Estradiol (E2) Mediated Secretion of Vascular Endothelial Growth Factor (VEGF) and Stimulation of T-Regulatory Cells (T-Regs): Their Role for the Worse Prognosis of Breast Cancer (BC) in Premenopause
Recchia F, Candeloro G, Desideri G, Necozione S, Recchia COC, Rea S. Civilian Hospital, Avezzano, AQ, Italy; University, L’Aquila, AQ, Italy.

P4-08-03  Serum Autoantibodies to Breast Cancer Associated Antigens Reflect Tumor Biology: An Opportunity for Early Detection & Prevention?
Eiermann W, Jackson L, Murray A, Chapman CJ, Peek LJ, Widschwendter P, Allen J, Graham H, Robertson J-F. Fauenklinik vom Rheinfelden, Rheinfelden, Germany; Frauenklinik Rheinfelden, Ludwigsfelde-Teltow, Germany; Frauenklinik Rheinfelden, Hildesheim, Germany; Ev. Krankenhaus, Ludwigsfelde-Teltow, Germany; Hospital Gelsenkirchen, Gelsenkirchen, Germany; Oncologianova, Recklinghausen, Germany; Gynäko-Onkologische Praxis Hannover, Hannover, Germany; Gynäkologische Gemeinschaftspraxis, Hildesheim, Germany; Ev. Krankenhaus, Ludwigsfelde-Teltow, Germany; Frauenklinik Rheinfelden, Rheinfelden, Germany.

P4-08-04  Preoperative Thrombin Pathway Activation Identifies Node Positive Patients in Early Breast Cancer
Shaker H, Kirwan C, Landberg G, Bundjor NJ. University Hospital of South Manchester, Manchester, United Kingdom; Paterson Institute for Cancer Research, Manchester, United Kingdom.

P4-08-05  Diagnostic Significance of Exosomal miRNAs in the Plasma of Breast Cancer Patients
Kwong A, Ng EKO, Leung CPH, Chan V, Li R, Wong CLP, Ma ESK. The University of Hong Kong,Pokfulam, Hong Kong, Hong Kong Sanatorium & Hospital, Hong Kong.

P4-09-01  Identification of Poor Prognosis T1T2N0 Luminal ERBB2-ve Breast Carcinomas

P4-09-02  G Protein-Coupled Estrogen Receptor 1 Positively Correlates with Estrogen Receptor α Expression and Increased Distant Disease-Free Survival of Breast Cancer Patients

P4-09-03  Clinical Significance of HER2+ and Triple-Negative Status in Patients with Tumor Size ≤ 1 cm and Node Negative Breast Cancer
Shao T, Boobil SK, Boache-Adjie K, Klein P. Beth Israel Medical Center, Continuum Cancer Centers of New York, New York, NY.

P4-09-04  Do Decreased CEP17 Signals Indicate the Presence of “Monosomy” in Breast Cancer? A Study of HER2 Gene Status and HER2 Immunohistochemistry
Tse CH, Hwang HC, Goldstein LC, Kandali SP, Duisterhoef SM, Kussick SL, Gown AM. PhenoPath Laboratories, Seattle, WA.

P4-09-05  CA15-3 Adds Prognostic Information in “Luminal” Type Breast Cancer - A Single Center Experience Evaluating 700 Patients with a Median Follow-Up of 5 Years
Thomssen C, Kettelhardt EJ, Vetter M, Steer S, Ruiter T, Holzhausen H-J, Strauß H-G, Görgle R, GYN/OB, Halle (Saale), Germany; Inst. of Pathology, Halle (Saale), Germany.

P4-09-06  The Prognostic Value of Tumour-Stroma Ratio in Triple Negative Breast Cancer
Moorman AM, Vink R, Heijmans HJ, van der Palen J, Kouwenhoven EA. Hospital Group Twente, Almelo, Netherlands; Pathology Laboratory East Netherlands, Enschede, Netherlands; Medical Spectrum Twente, Enschede, Netherlands; Hospital Group Twente, Hengelo, Netherlands.

P4-09-07  Breast Cancer Outcome by Combined Immunohistochemical ER/PR/HER2 Receptor Phenotype

P4-09-08  Secretary Leukocyte Protease Inhibitor as a Differential Diagnostic Biomarker of Tumor Emboli in Inflammatory Breast Cancer

P4-09-09  Expression of c-MET and Phospho-c-MET in Breast Cancers by Subtype and Its Impact on Survival Outcomes

P4-09-10  Assessment of Circulating Immune Parameters in Patients with Metastatic Breast Cancer Improves Survival Prediction
P4-09-11 Kinesin Family Member 2C (KIF2C) Is a New Surrogate Prognostic Marker in Breast Cancer (BC)
Abdel-Fatah TMA, Green AR, Lemetre C, Moseley P, Chan S, Ellis IO, Balls G. Nottingham University City Hospital NHS Trust, Nottingham, United Kingdom; Nottingham University, Nottingham, United Kingdom; Nottingham Trent University, Nottingham, United Kingdom.

P4-09-12 Clinical Validation of Immunohistochemical Signature Predictive of Patients’ 8 Year Outcome in Node Negative Breast Carcinomas
Charpin C, Tavassoli F, Giusiano S, Secq V, Villaret J, Garcia S, Lavaut M-N, Bonnier P, Birnbaum D, Iovanna J. INSERM U624 - Hospital Nord, Marseille, France; Yale University School of Medicine, New Haven; INSERM UMR 891, Marseille, France.

P4-09-13 External Validation of Adjuvant! Online Breast Cancer Prognosis Tool. Improvement Is Still Needed

P4-09-14 Differential Expression of the Akt1 Isoform in Mammary Ductal Carcinoma
Sanders AJ, Mansel RE, Jiang WG. Cardiff University School of Medicine, Cardiff, United Kingdom.

P4-09-15 Clinical and Pathological Predictors of Outcome among Patients with Triple Negative Breast Cancer
Pearlstone DB, Gray M, Garofalo RM, Trapani M, Nyirenda T, Hazelwood VA. Hackensack University Medical Center, Hackensack, NJ; Stevens Institute of Technology, Hoboken, NJ; John Theurer Cancer Center, Hackensack, NJ.

P4-09-16 Factors Affecting the Development of Axillary Lymph Node Metastases in T1A-T1B Breast Carcinomas
Khair TA, Boolbol SK, Boachi-Adjei K, Klein P. Beth Israel Medical Center, Continuum Cancer Centers of New York, New York, NY.

P4-09-17 Leptin and Adiponectin Expression in Breast Cancer
Jeong Y-J, Jeong H-Y, Bong J-G, Park S-H, Choi H-K. School of Medicine, Catholic University of Daegu, Daegu, Korea; College of Pharmacy, Kyung Hee University, Seoul, Korea.

P4-09-18 Australian Decision Impact Study: The Impact of Oncotype DX Recurrence Score (RS) on Adjuvant Treatment Decisions in Hormone Receptor Positive (HR+), Node Negative (NO) and Node Positive (N+) Early Stage Breast Cancer (ESBC) in the Multidisciplinary Clinic (MDC)
de Boer RH, Baker C, Speakman D, Mann B. Royal Melbourne Hospital, Melbourne, Victoria, Australia; Austin Hospital, Melbourne, Victoria, Australia; Peter MacCallum Cancer Institute, Melbourne, Victoria, Australia; Royal Melbourne and Royal Women’s Hospital, Melbourne, Victoria, Australia.

P4-09-19 PCNA+ Tumor Associated Macrophages Are Associated with M1 and M2 Gene Expression, and Confer Poor Prognosis in the Absence of Anti-Tumor Immune Environment

P4-09-20 Expression Profile of Interleukin 17B and the Receptor IL-17BR in Clinical Breast Cancer
Sanders AJ, Mansel RE, Mason MD, Jiang WG. Cardiff University School of Medicine, Cardiff, United Kingdom.

P4-09-21 A Novel Prognostic Marker for Triple-Negative Breast Cancers
Wallon UM, Sabol JL, Zsemba-Palko V, Carp NZ, Ciocca RM, Wojciechowski BS, Prendergast GC. Lankenau Institute for Medical Research, Wynnewood, PA; Lankenau Medical Center, Wynnewood, PA.

P4-09-22 Multicenter Quality Assurance Profile Review of Lobular Breast Carcinomas Versus the 21 Gene Recurrence Score: Assessment of Clinical Relevance
Dabbs DJ, Hicks D, Tubb R, Bhatta R, Bruksky A. University of Pittsburgh Medical Center, Pittsburgh, PA; University of Rochester Medical Center, Rochester, NY; Cleveland Clinic, Cleveland, OH.

P4-09-23 Monitoring Autophosphorylation of Mammalian Target of Rapamycin (mTOR) for Histoprognostic Grading of Invasive Breast Cancer: Impact on the Reclassification of Patients with Grade 2 Tumors Using the Nottingham Grading System
Sauri-Nadal T, Del Barco S, Vazquez-Martin A, Oliveras-Ferreros C, Cuff S, Martin-Castillo B, Lopez-Bonet E, Menendez JA. Catalun Institute of Oncology, Girona, Catalonia, Spain; Dr. Josep Trueta University Hospital, Girona, Catalonia, Spain.

P4-09-24 Correlation of Aurora Family Member Expression with Clinical Breast Cancer Prognosis
Annakesavan A, Sanders AJ, Harding KG. School of Medicine, Cardiff University, Cardiff, Wales, United Kingdom.

P4-09-25 Impact of Body Mass Index (BMI) for Clinical Outcomes in Japanese Breast Cancer Patients

P4-09-26 Prognostic Significance of Tissue Inhibitor of Metalloproteinase-1 (TIMP-1) in Breast Cancer
Dechaphunkul A, Phukaooul M, Kanjianapradit K, Graham K, Ghosh S, Santos C, Mackey JR. Cross Cancer Institute, University of Alberta, Edmonton, AB, Canada; Faculty of Medicine, Prince of Songkla University, Hat Yai, Songkhla, Thailand.

P4-09-27 Can We Predict the Benefit of the 70-Gene Signature in the Choice of Adjuvant Systemic Treatment for ER Positive, HER2 Negative Tumors in Daily Practice? A Single Institution Experience

P4-09-28 Comparison of Oncotype DX (ODX) and Mammastrat (MS) Risk Estimations and Correlations with Histologic Tumor Features in Low Grade, ER-Positive Invasive Breast Carcinoma (BC)
Acs G, Kilik J, Loftus L, Laronga C. Moffitt Cancer Center, Tampa, FL; Women’s Pathology Consultants, Ruffolo Hooper & Associates, Tampa, FL.

P4-09-29 A Nomogram To Predict Prognosis in Node-Negative Breast Carcinoma

P4-09-30 Relationship between DNA Ploidy, Biomarker Expression and Molecular Subtypes of Invasive Breast Cancer
Sarode VR, Rahardja D, Morrison DH, Peng Y, Rao R, Euhus D. University of Texas Southwestern Medical Center, Dallas, TX.

P4-09-31 Withdrawn

P4-09-32 Withdrawn

P4-09-33 The Prognostic Significance of Metaplastic Carcinoma of the Breast - A Comprehensive Comparison Study with Common Breast Cancers
Lai H-W, Kuo S-J, Chen D-R, Chen S-T. Changhua Christian Hospital, Changhua, Taiwan.
P4-09-34 Luminal Infiltrating Lobular Carcinoma of the Breast: Clinical and Prognostic Features

P4-09-35 Co-Expression of Ki-67 Is Critical for Outcome of Cyclooxygenase-2 Positive Breast Cancer

P4-10-01 Tumor Markers Predicting Recurrence Type after a Primary Ductal Carcinoma In Situ
Zhou W, Johansson C, Jeström K, Ringberg A, Blomqvist C, Amini R-M, Fälldiskog M-L, Wärnberg F. Uppsala University Hospital, Uppsala, Sweden; Malmö University Hospital, Malmö, Sweden; Lund University, Lund, Sweden; Helsinki University Central Hospital, Helsinki, Finland; Uppsala University, Uppsala, Sweden.

P4-10-02 A Meta-Analysis of the Association of Blood Levels of Vitamin-D and the Risk of Breast Cancer
Amir E, Carlsson L, Seruga B, Ocanà A, Goodwin P. Princess Margaret Hospital, Toronto, Canada; Institute of Oncology Ljubljana, Ljubljana, Slovenia; Albacete University Hospital, Albacete, Spain; Mount Sinai Hospital, Toronto, Canada.

P4-10-03 Association between BMI, Physical Activity and Breast Cancer Histologic Types
Nyante SJ, Dallal CM, Gierach GL, Sherman ME, Park Y, Hollenbeck AR, Brinton LA. National Cancer Institute, Rockville, MD; AARP, Washington, DC.

P4-10-04 Automated Breast Cancer Risk Assessment: Identifying High Risk Women in the Primary Care Setting
Ozanne E, Omer Z, Carlson K. University of California, San Francisco; Massachusetts General Hospital.

P4-10-05 Improved Breast Cancer Risk Assessment in Biopsied Women Using the Polyfactorial Model Oncovue®
Jupe ER, Pugh TW, Knowlton NS, DeFreese DC. InterGenetics LLC, Chotaw, OK.

P4-10-06 Evaluation of BRCAPro Risk Assessment Model in Patients with Ductal Carcinoma In Situ
Muse KI, Elsayegh N, Gutierrez-Barrera AM, Kuerer H, Valero V, Litton JK, Hortobagyi GN, Arun BK. UT MD Anderson Cancer Center, Houston, TX.

P4-10-07 Clinico-Pathologic Features of Breast Cancer Patients with Primary Metastatic Disease Versus Localized Disease: A Multicenter Study
Barinoff J, Hils R, Bender A, Gross J, Kurz C, Tauchert S, Mann E, Schwidde I, Ipsen B, Sawitzki K, Heitz F, Harper P, Traut A, du Bois M, A. Kliniken Essen Mitte, Essen, Germany; Dr.-Horst-Schmidt-Klinik, Wiesbaden, Germany; Asklepios Klinik, Lich, Germany; Sanikt Gertrauden-Krankenhaus, Berlin-Wilmersdorf, Germany; Klinikum Esslingen, Esslingen am Neckar, Germany; Cantinklinik St. Theresa, Saarbrücken, Germany; Universitätsklinikum Gießen und Marburg, Germany; Klinikum Frankfurt Höchst, Frankfurt am Main, Germany; Evangelisches Krankenhaus Wesel, Wesel, Germany.

P4-10-08 Risk Factor Profiles of Women with DCIS and Invasive Ductal Breast Cancers

P4-10-09 Hormone Replacement Therapy: A Benign Breast Disease’s Risk?

P4-10-10 Breast Cancer Risk Factors among Asian Versus Caucasian Women with BRCA1/2 Mutations
de Bruin MA, Kwong A, Goldstein BA, Lipson JA, Ikeda DM, McPherson LA, Sharma B, Kardashian A, Schackmann EA, Kingham KE, Mills MA, West DW, Ford JM, Kuiran AW. Stanford University School of Medicine, Stanford, CA; University of Hong Kong Li Ka Shing Faculty of Medicine, Hong Kong, China.

P4-10-11 Genetic and Environmental Predictors, Endogenous Hormones and Growth Factors and Risk of Estrogen Receptor-Positive Breast Cancer in Japanese Women
Yoshimoto N, Nishiyama T, Toyama T, Takahashi S, Shinaki N, Sugira H, Endo Y, Iwasa M, Fuji Y, Yamashita H. Nagoya City University Graduate School of Medical Sciences, Nagoya, Aichi, Japan.

P4-11-01 Bilateral Oophorectomy Is Associated with a Higher Prevalence of Arthritis and Lower Bone Mineral Density in Women 40 Years and Older
McCarthy AM, Visvanathan K. Johns Hopkins Bloomberg School of Public Health, Baltimore, MD; Johns Hopkins Sidney Kimmel Comprehensive Cancer Center, Baltimore, MD.

P4-11-02 Insulin-Like Growth Factor-1 (IGF-1), Insulin-Like Growth Factor Binding Protein-3 (IGFBP-3) and Lobule Type among Women in the Nurses’ Health Study II (NHS II)
Collins LC, Rice MS, Shen D, Connolly JL, Schnitt SJ, Tamimi RM. Beth Israel Deaconess Medical Center and Harvard Medical School; Brigham and Women’s Hospital and Harvard School of Public Health, Boston.

P4-11-03 Prognosis of Pregnancy-Associated Breast Cancer: A Meta-Analysis Involving 39,415 Patients
Azim, Jr HA, Santoro L, Russell-Edu W, Pentheroudakis G, Pavlidis N, Peccatoni FA. Institut Jules Bordet, Brussels, Belgium; European Institute of Oncology, Milan, Italy; University of Ioannina, Ioannina, Greece.

P4-11-04 Risk of Primary (PBC) and Contralateral Breast Cancer (CBC) after Ovarian Cancer (OC) in BRCA1 and BRCA2 Mutation Carriers: Implications for Surveillance and Risk Reducing Mastectomy

P4-11-05 Association between Bisphosphonate Use in Metastatic Breast Cancer (MBC) and Overall Survival
Mathew A, Mathew IE, Rozenszvig MQ, Bruyse AM. University of Pittsburgh Medical Center; University of Pittsburgh Cancer Institute; Magee-Womens Hospital.

P4-11-06 Uptake of Selective Estrogen Receptor Modulators and Other Breast Cancer Prevention Strategies among High-Risk Women Seen in a Breast Center
P4-11-07 Feasibility and Acceptability of Offering Breast Cancer Risk Estimation in the Context of the UK National Health Service Breast Cancer Screening Programme: A New Paradigm for Cancer Prevention

P4-11-08 Changes in the Distribution of Loco-Regional and Distant Breast Cancer Recurrences over the Last 20 Years: Implications for Patient Care and Future Research
Bouganim N, Clemons M, Amir E. The Ottawa Hospital Cancer Center, Ottawa, ON, Canada; Princess Margaret Hospital, Toronto, ON, Canada.

P4-11-09 Polymorphisms Related to Steroid Hormone Concentrations in Nipple Aspirate Fluid (NAF)

P4-11-10 Perceptions, Knowledge and Satisfaction with Contralateral Prophylactic Mastectomy among Young Women with Breast Cancer
Tracy MS, Meyer ME, Sepucha K, Gelber S, Hirshfield-Bartek J, Troyan S, Morrow M, Schapira L, Come S, Winer E, Partridge AH, Dana- Farber Cancer Institute, Boston, MA; Massachusetts General Hospital, Boston, MA; Memorial Sloan-Kettering Cancer Center, New York, NY; Beth Israel Deaconess Medical Center, Boston, MA.

P4-11-11 Thyroid Disease Is Associated with Breast Cancer: A Meta-Analysis
Hardefeldt PJ, Elick KD, Edirinne M. The University of Sydney, Sydney, NSW, Australia; Nepean Hospital, Pennth, NSW, Australia.

P4-11-12 Molecular Phenotype of Breast Cancers in a Large Cohort of Young Women According to Time Interval Since Pregnancy
Collins LC, Gelber S, Marothi JD, Cole KR, Kereakoglow S, Ruddy KE, Brachtel EF, Schapira L, Come SE, Borges VF, Schedin PJ, Warner E, Winer E, Partridge A. Beth Israel Deaconess Medical Center, Boston; Harvard Medical School, Boston; Dana Farber Cancer Institute, Boston; Dartmouth-Hitchcock Medical Center, Lebanon, Hanover, NH; Brigham and Women’s Hospital, Boston; Massachusetts General Hospital, Boston; University of Colorado Cancer Center, Colorado; Sunnybrook Odette Cancer Centre, Toronto.

P4-11-13 Influence of Two Years of Exemestane on Bone Mineral Density in Postmenopausal Women at Increased Risk of Developing Breast Cancer: a Companion Study to the NCIC CTG MAP.3 Trial
Goss PE, Richardson H, Ingle JN, Chlebowski RT, Fabian CJ, Garber JE, Sarto GE, Hiltz A, Tu D, Cheung AM. Massachusetts General Hospital Cancer Center, Boston, MA; Queen’s University, Kingston, ON, Canada; Mayo Clinic, Rochester, MN; Los Angeles Biomedical Research Institute, Torrance, CA; University of Kansas Medical Center, Westwood, KS; Dana Farber Cancer Institute, Boston, MA; Center for Women’s Health and Health Research, Madison, WI; General Hospital, Toronto, ON, Canada.

P4-11-14 Number Needed To Treat (NNT) as a Measure of Incremental Drug Benefit: Denosumab vs. Zoledronic Acid for the Prevention of Skeletal Related Events (SREs) in Advanced Breast Cancer
Dranitsaris G, Kaura S. Augmentum Pharma Consulting, Toronto, Canada; Novartis Pharmaceuticals, NJ.

P4-11-15 Increased Propensity of Triple Negative Breast Cancer (TNBC) in Premenopausal Patients after Exogenous Hormonal Intake (EHI)

P4-11-16 Withdrawn

P4-11-17 Noninferiority (NI) Phase III Trials in Advanced Breast Cancer (ABC) over 12 Years
Saad ED, Milindo MS. Dendrix Research, Sao Paulo, Brazil.

P4-11-18 Mammographic Surveillance in Atypical Hyperplasia of the Breast and Subsequent Development of Cancer. A Need for Long Term Follow Up
Korron R, Sridharan U, Mitchell G, Holcombe C. Royal Liverpool and Broadgreen University Hospital Trust, Liverpool, Merseyside, United Kingdom.

Yong M, Christiansen CF, Gammelager H, Svane C, Chia V, Atchison C, Fryzek J. Ameera Inc. Thousand Oaks, CA; Aarhus University Hospital, Aarhus, Denmark; Exponent, Alexandria, VA.

P4-11-20 Observational Study of Body Weight Changes and Metabolic Syndrome in Breast Cancer Patients Receiving Adjuvant Therapy: Characteristics of Dietary Pattern in Korean Breast Cancer Patients

P4-11-21 A Retrospective Analysis of Women at Increased Lifetime Risk for Breast Cancer: Referral Patterns to Subspecialty Providers, Recommendations and Outcomes

P4-11-22 Familial History of Cancer among Breast Cancer Women under 36 yr. in Rio De Janeiro, Brazil
Koifman S, Ortega GPJ, Koifman RJ. National School of Public Health, Oswaldo Cruz Foundation (FIOCRUZ), Rio de Janeiro, RJ, Brazil.

P4-11-23 Lifelong exposure to isoflavones results in a reduced responsivity of the mammary gland towards estradiol
Diel P, Vollmer G, Hertrampf M, Moller FJ, Scholtens D, Molzberger A. German Sports University, Cologne, Germany; Technical University Dresden, Dresden, Germany; Max Rubner-Institut, Karlsruhe, Germany.

Psychosocial, Quality of Life, and Educational Aspects: Survivorship Research

P4-12-01 The Breast-Activity and Healthy Eating after Diagnosis (B-AHEAD) Study - A Randomised Comparison of Weight Control Programmes during Adjuvant Treatment
Harvie M, Pegington M, Bundre N, Campbell A, Wolstenholme J, Adams J, Speed S, Morris J, Howell A. University Hospital of South Manchester, Manchester, United Kingdom; University of Dundee, United Kingdom; University of Oxford, United Kingdom; University of Manchester, United Kingdom; University Hospital of South Manchester, United Kingdom.

P4-12-02 The Association of Anastrozole Adherence and Cognitive Function in Breast Cancer
Sereika SM, Dunbar-Jacob JM, Ryan CM, Adam B, Bender CM. University of Pittsburgh, Pittsburgh, PA; University of Pittsburgh Medical Center, Pittsburgh, PA.
P4-12-03 Post-Diagnosis Weight Gain in Breast Cancer Survivors: When Should We Intervene? Brayshaw PT, Cleveland RJ, Stevens J, Rosamond W, Abrahamson PE, Teitelbaum SL, Neugut AI, Gammon MD. University of North Carolina at Chapel Hill, Chapel Hill, NC, Mount Sinai School of Medicine, New York, NY; Columbia University, New York, NY.

P4-12-04 Withdrawn

P4-12-05 Impact of the Lifestyle Intervention Study in Adjunct Treatment of Early Breast Cancer (LISA) Weight Loss Intervention upon Physical Activity Ligibel JA, Segal R, Pond G, D’Amico M-J, Pichardt KJ, Levine M, Goodwin PJ, Dana-Farber Cancer Institute, Boston, MA; University of Ottawa, Ottawa, ON, McMaster University, Hamilton, ON, University of Toronto, Toronto, ON.

P4-12-06 Risk Factors for Relative Weight Gain >10% in Breast Cancer Survivors: Findings from the SU VI.MAX Cohort Zelek L, Czernichow S, Galan P, Hercberg S. Assistance Publique Hôpitaux de Paris, CHU Avicenne, Bobigny, France; INSERM U1125 INRA/CNAM/Université Paris 13, Bobigny, France.

P4-12-07 Outcomes of a Behavioral Weight Control Intervention among Rural Breast Cancer Survivors Befort CA, Klemp JR, Austin HL, Krigel S, Sullivan DK, Schmitz KH, Perri MG, Fabian CJ. University of Kansas Medical Center, University of Pennsylvania; University of Florida.

P4-12-08 Five Year Preliminary Outcomes of a Prospective Surveillance Model To Reduce Upper Extremity Morbidity Related to Breast Cancer Treatment Stout NL, Pfalter L, Levy E, McGarvey C, Gerber L, Springer B, Soballe P. National Naval Medical Center, University of Michigan-Flint; National Institutes of Health; CLM Consulting. George Mason University; Office of the Surgeon General; Naval Hospital San Diego.

P4-12-09 Withdrawn

P4-12-10 A Prospective Study of Physical Activity Patterns and Changes in Breast Cancer Patients during Active Breast Cancer Treatment Kim I-R, Choi E-K, Nam S-J, Lee J, Lee S-K, Yang J, Noh DY, Han W, Cho J. Samsung Comprehensive Cancer Center, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea; Surgery-Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea; Konkuk University Medical Center, Konkuk University School of Medicine, Seoul, Korea; Seoul National University Hospital, Seoul National University School of Medicine, Seoul, Korea; Johns Hopkins Bloomberg School of Public Health, Baltimore; Behavior and Society, Johns Hopkins Bloomberg School of Public Health, Baltimore.

P4-12-11 Myelodysplastic Syndrome Post Primary Breast Cancer Treatment: Cases from a Community Cancer Center: 1990-2010 Kaplan HG, Malmgren JA, Atwood MK. Swedish Cancer Institute, Seattle, WA; HealthStat Consulting, Inc., Seattle, WA.

P4-12-12 Patient-, Illness-, and/or Treatment-Related Baseline Predictors of Nonadherence to Oral Hormonal Therapy Wickersham KE, Sereika SM, Bender CM. University of Pittsburgh, Pittsburgh, PA.


P4-12-14 Pilot Study Utilizing Fluorine-18 Fluorodeoxyglucose (F-18 FDG) Positron Emission Tomography-Computed Tomography Scan (PET-CT Scan) To Investigate Brain Metabolic Changes during Treatment in Women with Breast Cancer Virani S, Lagos R, Hobbs G, Marano G, Nagaiah G, Abraham J. West Virginia University, Morgantown, WV.

P4-12-15 Cancer Survivor Care: An Evaluation of a Group Visit Model of Care for Breast Cancer Survivors at Duke Cancer Center Trotter KJ, Schneider S. Duke University Medical Center, Durham, NC; Duke University, Durham, NC.

P4-13-01 Pain Severity and Analgesic Use Associated with Skeletal-Related Events in Patients with Advanced Breast Cancer and Bone Metastases Fallowfield L, Cleeland CS, Body J-J, Stopeck A, von Moos R, Patrick DL, Clemens M, Tonkin K, Masuda N, Lipton A, De Boer R, Salvagni S, Toselio Olivera C, Ying W, Braun A, Cong Z. Cancer Research UK, University of Sussex, Brighton, United Kingdom; University of Texas, M.D. Anderson Cancer Center, Houston, TX; CHU Brugmann, Université Libre de Bruxelles, Brussels, Belgium; University of Arizona, Arizona Cancer Center, Tucson, AZ; Kantonsspital Graubünden, Chur, Switzerland; University of Washington, Seattle, WA; The Ottawa Hospital Cancer Centre, Ottawa, ON, Canada; Cross Cancer Institute, Edmonton, AB, Canada, Osaka National Hospital, Osaka, Japan; Penn State Milton S. Hershey Medical Center, Hershey, PA; Royal Melbourne Hospital, Melbourne, Australia; Azienda Ospedaliera di Parma, Parma, Italy; Instituto Brasileiro de Controle do Cancer-IBCC, São Paulo, Brazil; Amgen Inc., Thousand Oaks, CA.

P4-13-02 Hospice Utilization and End of Life Care in Metastatic Breast Cancer Patients at a Comprehensive Cancer Center O’Connor TL, Ngamphaiboon N, Morris J, Callinan NK, Milch RA, Kerr CH, Watroba N, Edge SB. Roswell Park Cancer Institute, Buffalo, NY; The Buffalo Center for Hospice and Palliative Care, Cheektowaga, NY.

P4-13-03 Retrospective Analysis of Palonosetron Compared with Older 5-HT3 Receptor Antagonists (Ondansetron, Dolasetron, and Granisetron) in Preventing Anthracycline-Induced Nausea and Vomiting in Breast Cancer Patients from Four Phase 3 Trials Rugo HS, Schwartzberg L, Morow GR, Barbour SY, Ballinari G, Thorn MD, Cox D. UCSF Helen Diller Family Comprehensive Cancer Center, West Clinic; University of Rochester, School of Medicine and Dentistry; Duke University Medical Center, Helsinn Healthcare SA; Statistical Resources Inc; Eisai, Inc.

P4-13-04 Psychosocial, Quality of Life, and Educational Aspects: Palliation and Pain Management

P4-14-01 Weight-Adjusted Change of Unilateral Arm Volumes for Quantification of Lymphedema after Bilateral Breast Surgery Miller CI, Specht MC, Ancukiewicz M, Skolny MN. O‘Toole J, Taghian AG. Massachusetts General Hospital, Boston, MA.

P4-14-02 Risk Factors for Early Lymphoedema Do not Predict Late Development of Lymphoedema Kilbreath SL, Beith J, Refshauge KM, Lee M-J, Ward LC. University of Sydney, Sydney, NSW, Australia; Royal Prince Alfred Hospital, Camperdown, NSW, Australia; University of Queensland, St Lucia, QLD, Australia.

P4-14-03 Support Groups in Breast Cancer: An Evidence Based Assessment of 1606 Patients with Concerning Topics for Support Group Discussion and Presentation Gralla RJ, Morse KD, Rittenberg CN, Petersen JA, Rosen LM, Lesser M. Hofstra North Shore - LIJ School of Medicine, New York, NY; Nexeura, Seattle, WA; Feinstein Institute for Medical Research, Manhasset, NY.

Chemotherapy-Induced Alopecia, Body Image and Psychological Distress in Women with Breast Cancer: A Prospective Study
Choi E-K, Kim I-R, Nam S-I, Lee J, Yang J, Lee S-K, Noh D-Y, Han W, Cho J. Samsung Comprehensive Cancer Center, Samsung Medical Center, Sungkyunkwan University School of Medicine; Johns Hopkins Bloomberg School of Public Health; Samsung Medical Center, Sungkyunkwan University School of Medicine; Konkuk University Medical Center, Konkuk University School of Medicine; Seoul National University Hospital, Seoul National University School of Medicine.

Psycosocial, Quality of Life, and Educational Aspects: Psychological Aspects

High Prevalence of Prospective Memory (PM) Impairment in Early Breast Cancer (EBC) Survivors within 1 Year of Adjuvant Chemotherapy Completion: Novel Findings Concerning Post Chemotherapy Cognitive Effects
Paquet L, Verma S, Collins B, Song X, Wheatley-Price P, Hopkins S, Segal R, Dent S, Mirkys D, Goel R, Young V, Clemons M, Keller O, Chinneck A, Young R, Bedard M. Carleton University, Ottawa, ON, Canada; The Ottawa Hospital Cancer Center, Ottawa, ON, Canada; The Ottawa Hospital, Ottawa, ON, Canada.

Clinical and Epidemiological Correlates of Elevated Distress Thermometer Scores in Breast Cancer Patients
Powers K, Pappas L, Buchmann L, Anderson L, Gauchay L, Rich A, Agarwali J. University of Utah, Salt Lake City, UT; Huntsman Cancer Institute, Salt Lake City, UT.

Patient-Provider Communication and Patient Informational Needs for Breast Reconstruction Post-Mastectomy: Results from a National Survey

Proteomic Analysis of Patient Plasma Identifies Parathyroid Hormone Related Protein (PTHrP12-48) as a Potential Biomarker of Breast Cancer Bone Metastasis
Hasham CL, Byrum SD, Leitell K, Suhail AM, Lipion A, Suva LJ, Winthrop P. Rockefeller Cancer Institute, University of Arkansas for Medical Sciences, Little Rock, AR; Penn State Hershey Cancer Institute, Penn State Hershey Medical Center, Hershey, PA; Lebanon VA Medical Center, Lebanon, PA.

Problems with Identifying Bone Metastasis-Specific Genes without Considering Biological Differences between ER-Positive and ER-Negative Breast Cancers
Hayashi N, Iwamoto T, Qiu Y, Nikura N, Sanatpia L, Nakamura S, Hortobagyi GN, Pusztai L, Symmans F, Leno NT. The University of Texas MD Anderson Cancer Center, Houston, TX; Hospital of Prato, Prato, Italy; Instituto Toscano Tumori, Prato, Italy; Showa University School of Medicine, Tokyo, Japan.

Proof of the Anti-Tumour Effect of Zoledronic Acid (ZA) in Naive Bone-Only Metastatic and Locally Advanced Breast Cancer: Results from the Biological Window Therapy
Foroni C, Andreis D, Maldotti M, Cappelletti MR, Generali DG. Istituti Ospitalieri di Cremona, Cremona, Italy.

An Open-Label, Phase IIa, Non-Randomized Study of Radium-223 in Breast Cancer Patients with Bone Dominant Disease No Longer Considered Suitable for Endocrine Therapy
Coleman R, Flamen P, Naume B, Jerusalem G, Garcia C, Piccart M, O’Bryan-Tear CG, Aknes A-K. Weston Park Hospital, Sheffield, United Kingdom; Institut Jules Bordet, Brussels, Belgium; Oslo University Hospital, Oslo, Norway; CHU Sart Tilman, B3S, Liege, Belgium; Algeta ASA, Oslo, Norway; Bayer Healthcare Pharmaceuticals, Montville, NJ.

A Randomized Phase 2 Study of a Loading Dose of Ibandronate in Patients with Bone Metastases from Breast Cancer
Ritchie DM, Bray C, Canney P. Beatson West of Scotland Cancer Centre, Glasgow, United Kingdom.

Expression Patterns of Receptor Activator of Nuclear Factor-κB (RANK) and Src in a Series of Primary Breast Tumors (BT) and Bone Metastases (BM) in Patients (pts) with Metastatic Breast Cancer (MBC)
Gucalp A, Comen E, Redana S, Evangelista L, Giri DD, Zhang XH, Patil S, Akram M, Norton L, Hudes CA, Forner MN. Memorial Sloan-Kettering Cancer Center, New York, NY; Sloan-Kettering Institute, New York, NY; Piemontese per l’Oncologia - Institute for Cancer Research and Treatment, Candiolo, Italy; Istituto Oncologico Veneto (IOV - IRCCS), Padua, Italy.

Breast Cancer Bone Metastasis Prevalence and Survival Outcomes: A Quantitative Review of the Literature

Pilot Randomized Trial of De-escalated (q12 Weekly) Versus Standard (q3-4 Weekly) Intravenous Bisphosphonates in Women with Low-Risk Bone Metastases from Breast Cancer
Amir E, Freedman O, Carlsson L, Usman T, Lee E, Dranitsaris G, Clemons M. Princess Margaret Hospital and The University of Toronto, Canada; The Ottawa Hospital Cancer Centre and The University of Ottawa, Canada.

Health Resource Utilization (HRU) Associated with Skeletal-Related Events (SREs) in Advanced Breast Cancer Patients with Bone Metastases: Results from a Prospective Multinational Observational Study
Lutfner D, Lorusso V, Duren I, Hechmati G, Garzon-Rodriguez C, Ashcroft J, Bahl A, Ghelani P, Wei R, Thomas E, Hoefele H. Universitatsmedizin Berlin, Berlin, Germany; Oncology Institute ASL, Leeece, Italy; Centro Integral Oncologico Clara Campal (CIOC), Madrid, Spain; Amgen (Europe) GmbH, Zug, Switzerland; Instituto Catalán Oncología ICO-IDIBELL, Barcelona, Spain; Pinderfields General Hospital, Wakefield, United Kingdom; University Hospitals Bristol, Bristol, United Kingdom; Ovatech Solutions, London, United Kingdom; Amgen Inc., So. Francisco, CA; Forschungszentrum Ruhr, Witten, Germany.

Trastuzumab Does Not Increase the Incidence of Central Nervous System (CNS) Relapses in HER2-Positive Early Breast Cancer: The HERA Trial Experience

Whole-Brain Radiation Therapy Plus Concomitant Temozolomide for the Treatment of Brain Metastases from Breast Cancer: A Randomized Prospective Multicenter Phase II Study

Incidence Rate of Asymptomatic Brain Metastases in Patients with HER2+ Metastatic Breast Cancer Screened for EGFR111438/CEREBEL Study
Pivot X, Hackmann J, Manikhas A, Moore Y, Parikh R, Krohri D, Joshi A, Aktan G, Coleman R. University Hospital of Besancon; Marien-Hospital, St Petersburg City Clinical Oncology Dispensary; GlaxoSmithKline, Collegeville, PA; GlaxoSmithKline, Uxbridge, United Kingdom; Weston Park Hospital.
Treatment – Types and Sites of Breast Cancer: Inflammatory Breast Cancer

P4-20-01 Multicentric Phase II PACS 09/Beverly1 Trial: First Efficacy and Safety Results of Neoadjuvant Chemotherapy Combined with Bevacizumab in HER2-Negative Patients with Non-Metastatic Inflammatory Breast Cancer
Viens P, Petit T, Dalenc F, Pienga JY, Delcoeur T, Romieu G, Bonneterre J, Ferrero J-M, Kerbrat P, Mouret-Reynier M-A, Bachelot T, Soulie P, Leebourrs F, Fymard J-C, Deblock M, Lortholary A, Hardy-Bessard A-C, Boher J-M, Asselain B, Charafe-Jauffret E, Lemonsin J, Martin A-L, Andre F. Institut Paoli Calmettes, Marseille, France; Centre Paul Strauss, Strasbourg, France; Institut Claudius Regaud, Toulouse, France; Institut Curie, Paris, France; Centre François Baclesse, Caen, France; Centre Val d’Aurelle, Montpellier, France; Centre Oscar Lambret, Lille, France; Centre Antoine Lacassagne, Nice, France; Centre Eugène Marquis, Rennes, France; Centre Jean Perrin, Clermont-Ferrand, France; Centre Leon Berard, Lyon, France; Centre Paul Papan, Angers, France; Institut Curie, Saint Cloud, France; Institut Jean Godinot, Reims, France; Centre Alexis Vautrin, Nancy, France; Centre Catherine de Sienne, Nantes, France; Clinique Armoiricaine, Saint Brieuc, France; B&d Oncancer, Paris, France; Institut Gustave Roussy, Villejuif, France.

P4-20-02 Inflammatory Breast Cancer (IBC) in the National Comprehensive Cancer Network (NCCN): The Disease, the Recurrence Pattern and the Outcome
Lubbe W, Li T, Hughes M, Ottesen R, Cristofanilli M, Weeks J, Wong Y-N. Fox Chase Cancer Center, Philadelphia, PA; Dana-Farber Cancer Institute, Boston, MA; City of Hope Cancer Center, Duarte, CA.

P4-20-03 T-Cell Cytokine Production Related to Progression of Breast Cancer Patients
Cohen EN, Gao H, Lee B-N, Giordano A, Tin S, Anfossi S, Parker CA, Cristofanilli M, Valero V, Alvarez RH, Hortobagyi GN, Woodward WA, Ueno NT, Reuben JM. The University of Texas MD Anderson Cancer Center, Houston, TX; Fox Chase Cancer Center, Philadelphia, PA; The University of Texas Graduate School of Biomedical Sciences at Houston, Houston, TX.

P4-20-04 Cytokine Synthesis by Activated Dendritic Cells in Relation to Disease Progression in Inflammatory Breast Cancer (IBC)
Gao H, Cohen EN, Lee B-N, Giordano A, Tin S, Anfossi S, Parker CA, Cristofanilli M, Valero V, Alvarez RH, Hortobagyi GN, Woodward WA, Ueno NT, Reuben JM. The University of Texas MD Anderson Cancer Center, Houston, TX; Fox Chase Cancer Center, Philadelphia, PA.

P4-20-05 Inflammatory Breast Cancer: Comparison of Epidemiology, Biology, and Prognosis between Japan and the United States, a Hospital-Based Study
Yamauchi H, Natori A, Hayashi N, Soejima K, Takahashi O, Fukui T, Nakamura S, Cristofanilli M, Ueno N. St. Luke’s International Hospital, Chuo-ku, Tokyo, Japan; Showa University, Shinagawa-ku, Tokyo, Japan; Fox Chase Cancer Center, Philadelphia, PA; The University of Texas MD Anderson Cancer Center, Houston, TX.

7:00 am–9:00 am
POSTER DISCUSSION VII: WHAT’S NEW IN CHEMOTHERAPY TRIALS?
Ballroom A

Viewing 7:00 am
Discussion 7:45 am

Discussant: Clifford Hudis, MD
Memorial Sloan-Kettering Cancer Center
New York, NY

PD07-01 Sequential Treatment with Epirubicin/Cyclophosphamide, Followed by Docetaxel vs. FEC120 in the Adjuvant Treatment of Breast Cancer Patients with Extensive Lymph Node Involvement: Final Survival Analysis of the German ADEBAR Phase III Study
Janni WJ, Harbeck N, Sommer H, Rack B, Salmen J, Augustin D, Simon W, Jueckstock J, Wischum N, Arkeke K, Melcher C, Friese K, Kechle M. Heinrich-Heine-University, Dusseldorf, Germany; University Cologne, Cologne, Germany; Ludwig-Maximilians-University, Munich, Germany; Mammazentrum Ostbayern, Deggendorf, Germany; Robert-Bosch-Krankenhaus, Stuttgart, Germany; Zentalklinikum, Augsburg, Germany; Technische Universität, Munich, Germany.

PD07-02 Docetaxel Is Superior to Paclitaxel Given Every Three Weeks in Post Operative Patients with Node-Positive Breast Cancer: Results of the Final Analyses of the NSAS-BC (National Surgical Adjuvant Breast Study of Cancer) 02 Trial from Japan
Watanabe T, Kuranami M, Inoue K, Masuda N, Aogi K, Iwata H, Mukai H, Uemura Y, Ohashi Y. Hamamatsu Oncology Center, Hamamatsu, Japan; Kitasato University Hospital, Sagamihara, Japan; Saitama Cancer Center, Inachou-омuro, Japan; NHO Osaka National Hospital, Osaka, Japan; Shikoku Cancer Center, Matsuyama, Japan; Kyushu Cancer Center, Fukuoka, Japan; Aichi Cancer Center, Nagoya, Japan; National Cancer Center, Kashiwa, Japan; University of Tokyo, Tokyo, Japan.

PD07-03 Phase II Trial of Adjuvant TC (Docetaxel/Cyclophosphamide) Plus Trastuzumab (HER TC) in HER2-Positive Early Stage Breast Cancer Patients
Jones S, Collea R, Paul D, Oratz R, Sedlacek S, Favret AM, Gore, Jr II, Lindquist DL, Holmes FA, Allison MAK, Steinberg MS, Stokoe C, Portillo RM, Crockett M, Wang Y, Asmar L, Robert N, O’Shaughnessy J. US Oncology, The Woodlands, TX; Baylor Sammons Cancer Center, Dallas, TX; New York Oncology Hematology, Albany, NY; Rocky Mountain Cancer Center, Denver, CO; Physician, New York, NY; Virginia Cancer Specialists, PC, Fairfax, VA; Birmingham Hematology and Oncology, Birmingham, AL; Arizona Oncology Associates, Sedona, AZ; Texas Oncology, Houston, TX; Comprehensive Cancer Center, Henderson, NV; Virginia Oncology Associates, Virginia Beach, VA; Texas Oncology, Plano, TX; Texas Oncology, El Paso, TX.

PD07-04 Lapatinib vs Trastuzumab in Combination with Standard EC-D Chemotherapy in the Neoadjuvant Treatment of HER2+ Patients. Results from the GECAM 2006-14 Phase II Randomized Trial
Alba E, Albanell J, de la Haba J, Barnadas A, Calvo L, Sanchez P, Ramos M, Rojo F, Burgués O, Porrás I, Tibau A, CArrasco E, Cámara MC, Lluch A. Hospital Virgen de la Victoria, Malaga, Spain; Hospital del Mar, Barcelona, Spain; Hospital Universitario Reina Sofia, Córdoba, Spain; Hospital Santa Creu i Sant Pau, Barcelona, Spain; Complejo Hospitalario Universitario de A Coruña, A Coruña, Spain; Hospital General de Jaén, Jaén, Spain; Centro Oncológico de Gaúlica, A Coruña, Spain; Fundación Jiménez Díaz, Madrid, Spain; Hospital Clínico Universitario de Valencia, Valencia, Spain; GECAM (Spanish Breast Cancer Research Group), San Sebastián de los Reyes, Madrid, Spain.
PD07-05 Local Recurrence Risk in 6377 Patients with Early Breast Cancer Receiving Neoadjuvant Anthracycline-Taxane +/- Trastuzumab Containing Chemotherapy

PD07-06 Adjuvant Chemotherapy with or without Darbepoetin alpha in Node-Positive Breast Cancer: Survival and Quality of Life Analysis from the Prospective Randomized WSG ARA Plus Trial
Nitz U, Gluz O, Oberhoff C, Reimer T, Schumacher C, Hackmann J, Warm M, Ulher C, Runde V, Kuemmel S, Zuna I, Harbeck N. West German Study Group, Moenchengladbach, Germany; Bethesda Hospital, Moenchengladbach, Germany; Bethesda Hospital, Wuppertal, Germany; University Hospital Essen, Essen, Germany; Catholical Hospital Essen North, Essen, Germany; University Hospital Suedstadt, Rostock, Germany; St. Elisabeth Hospital, Cologne, Germany; Menen-Hospital Witten, Witten, Germany; University Hospital Cologne, Cologne, Germany; Krankenhaus Koeln-Holweide, Cologne, Germany; Gynecological Practice, Hildesheim, Germany; Wilhelm-Anton-Hospital Goeh, Goeh, Germany; Hospital Essen-Mitte, Essen, Germany.

PD07-07 Combination of Paclitaxel and Bevacizumab without or with Capecitabine as First-Line Treatment of HER2-Negative Locally Recurrent or Metastatic Breast Cancer (LR/MBC): First Results from a Randomized, Multicenter, Open-Label, Phase II Study of the Dutch Breast Cancer Trials’ Group (BOOG)
Lam SW, de Groot SM, Honkopes AH, Jager A, ten Tije AJ, Bos MMEM, Linn SC, van den Bosch J, Nortier JW, Braun JJ, de Graaf H, Portielje JEA, Los M, Gooyer DD, van Tinteren H, Boven E. VU University Medical Center, Amsterdam, Netherlands; Comprehensive Cancer Centre the Netherlands, Netherlands; Isala Clinics, Zwolle, Netherlands; Erasmus Medical Center-Daniel den Hoed Cancer Center, Rotterdam, Netherlands; Tergooi Hospitals, Hilversum, Netherlands; Reinier de Graaf Hospital, Delft, Netherlands; The Netherlands Cancer Institute-Antoni van Leeuwenhoek Hospital, Amsterdam, Netherlands; Albert Schweitzer Hospital, Dordrecht, Netherlands; Leiden University Medical Center, Leiden, Netherlands; Vlietland Hospital, Schiedam, Netherlands; Medical Center Leeuwarden, Leeuwarden, Netherlands; Haga Hospital, The Hague, Netherlands; St. Antonius Hospital, Nieuwegein, Netherlands; Franciscus Hospital, Roosendaal, Netherlands.

PD07-08 The Effect on Surgical Complications of Bevacizumab Added to Neoadjuvant Chemotherapy: NSABP Protocol B-40
Bret HD, Tang G, Rastogi P, Geyer CE, André R, Atkins JN, Baez-Diaz L, Brufsky AM, Mehta RS, Fehrenbacher L, Pajon ER, Senecal FM, Gaur R, Mangolese RG, Adams PT, Gross HM, Costantino JP, Swan SM, Mamounas EP. Wolmark N. National Surgical Adjuvant Breast and Bowel Project (NSABP), Virginia Commonwealth University, Massey Cancer Center, University of Pittsburgh Graduate School of Public Health; University of Pittsburgh Cancer Institute School of Medicine; Allegheny General Hospital; Centre Hospitalier de l’Université de Montréal; Southeast Cancer Control Consortium CCOP, San Juan MBCCOP; University of Pittsburgh/Magee Women’s Hospital, University of California at Irvine, School of Medicine, Chao Family Comprehensive Cancer Center, Kaiser Permanente, Northern California; CCOP, Colorado Cancer Research Program; CCOP, North-West Medical Specialties; Kansas City Clinical Oncology Program; Jewish General Hospital, McGill University; Genesys Regional Medical Center, Dayton CCOP, Washington Cancer Institute, Washington Hospital Center; Aultman Health Foundation.

PD08-01 JNK2 Regulates Mammary Lineage Differentiation in Tumors and Normal Glands through Notch1 and p53
Cantrell MA, Ebelt ND, Van Den Berg CL. University of Texas at Austin, Austin, TX.

PD08-02 Targeting BCL-2 Expressing Breast Tumors with BH3-Mimetics - A New Class of Drugs in Breast Cancer?
Lindeman GJ, Oakes SR, Vailliant F, Lim E, Lee L, Breslin K, Feleppa F, Deb S, Ritchie ME, Takano E, Ward T, Fox SB, Generali D, Smyth GK, Strasser A, Huang DCS, Visvader JE. The Walter and Eliza Hall Institute of Medical Research, Parkville, VIC, Australia; The Royal Melbourne Hospital, Parkville, VIC, Australia; The University of Melbourne, Parkville, VIC, Australia; Peter MacCallum Cancer Centre, East Melbourne, VIC, Australia; Ospitaleri di Cremona, Cremona, Italy.

PD08-03 Inhibition of MEK/ERK- and JNK-Dependent Expression of Interleukin-6 and Interleukin-8 Targets Basal-Like Breast Cancer Stem Cells
Balko JM, Cook RS, Kuba MG, Miller TW, Bhola NE, Sanders ME, Meszoely IM, Dowsett M, Gomez H, Arteaga CL. Vanderbilt University, Nashville, TN; Royal Marsden Hospital, United Kingdom; Instituto Nacional de Enfermedades Neoplásicas, Lima, Peru.

PD08-04 Inhibition of the TGFβ/TGFβR2 Pathway Prevents Enrichment of Drug-Resistant Breast Cancer Stem Cells by Anti-Cancer Chemotherapy
Bhola N, Arteaga C. Vanderbilt University Medical Center, Nashville, TN.

PD08-05 Stat3 Signaling in Human Breast Cancer Stem Cells
Wei W, Zhang M, Tweardy D, Rosen J, Lewis M. Baylor College of Medicine.

PD08-06 ERK2 Promotes Stem Cell-Like Characteristics in Triple-Negative Breast Cancer
Bartholomeusz C, Saso H, Dadabin A, Kazuharu K, Hortobagyi GN. The University of Texas MD Anderson Cancer Center, Houston, TX.
PD08-07  Wound-Healing Drainage Fluids Promote Triple Negative Breast Cancer Progression

PD08-08  Preclinical Efficacy of the Combination of Met and Src Family Kinase Inhibitors in Triple-Negative Breast Cancer
Gartner EM, Kim EHH, Choi L, Boerner J. Karmanos Cancer Center at Wayne State University, Detroit, MI.

PD08-09  PTPN12 Gene Expression Signature in Triple Negative Breast Cancer Cohort
Ghazalpour A, Bender RP, McGinnis MJ, Ashfaq R. Caris Life Sciences, Phoenix, AZ.

PD08-10  High Frequency of Triple Negative Mammary Carcinomas in the Dog as Model of Human Breast Cancer

PD08-11  Targeting Porcupine, a Critical Node for Wnt Signalling in Cancer

9:00 am–9:30 am  PLENARY LECTURE 3
Exhibit Hall D

Advances in Axillary Surgery: Sentinel Nodes and Beyond
Barbara L. Smith, MD, PhD
Massachusetts General Hospital
Boston, MA

9:30 am–11:30 am  GENERAL SESSION 5
Exhibit Hall D
Moderator: Muthaffar Rimawi, MD
Baylor College of Medicine
Houston, TX

9:30  SS-1. Neoadjuvant pertuzumab and trastuzumab: Biomarker analyses of a 4-arm randomized phase II study (NeoSphere) in patients with HER2-positive breast cancer
Gianni L, Bianchini G, Kiermaier A, Bianchi G, Im Y-H, Pienkowski T, Roman L, Liu M-C, Tseng L-M, Ratnayake J, Szaa T, Ross GA, Valagusa P. Oncologia Medica, San Raffaele Cancer Center, Milan, Italy; Roche, Basel, Switzerland; Oncologia Medica 1, Fondazione IRCCS Instituto Nazionale Tumori, Milan, Italy; Samsung Medical Center, Seoul, Korea; Centrum Onkologii, Warsaw, Poland; Leningrad Regional Oncology Dispensary, St Petersburg, Russian Federation; Koo Foundation Sun Yat-Sen Cancer Center, Taipei, Taiwan; Taipei-Veterans General Hospital, National Yang-Ming University, Taipei, Taiwan; Roche Products Limited, Welwyn, United Kingdom; Genentech, South San Francisco, CA; Fondazione Michelangelo, Milan, Italy.

9:45  SS-2. PAM50 HER2-enriched subtype enriches for tumor response to neoadjuvant anthracyclines/taxane and trastuzumab/taxane containing regimens in HER2-positive breast cancer
Cheang MCU, Prat A, Fan C, Perou CM. Lineberger Comprehensive Cancer Center, Chapel Hill, NC.

10:00  SS-3. Basement membrane localized tumor cells are protected from PARP-1 targeted therapy in vivo
Zoeller JJ, Bronson RT, Gilmer TM, Selfers LM, Lu Y, Mills GB, Brugge JS. Harvard Medical School, Boston, MA; GlaxoSmithKline, Research Triangle Park, NC; UT MD Anderson Cancer Center, Houston, TX.

10:15  SS-4. pCR as a surrogate in HER2-positive patients treated with trastuzumab

10:30  SS-5. A phase III, randomized, double-blind, placebo-controlled registration trial to evaluate the efficacy and safety of pertuzumab + trastuzumab + docetaxel vs. placebo + trastuzumab + docetaxel in patients with previously untreated HER2-positive metastatic breast cancer (CLEOPATRA)
Baselga J, Kim S-B, Im S-A, Hegg R, Im Y-H, Roman L, Pedrini JL, Cortés J, Knott A, Clark E, Ross GA, Swain SM. Massachusetts General Hospital Cancer Center, Boston, MA; Asan Medical Center, University of Ulsan, College of Medicine, Seoul, Korea; Seoul National University College of Medicine, Seoul, Korea; Hospital Pêrola Byington, São Paulo, Brazil; Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea; Lengrad Regional Oncology Dispensary, St. Petersburg, Russian Federation; CPMEC-Mastology Unit of Consejoacion Hospital, Porto Alegre, Brazil; Vall d’Hebron University Hospital, Barcelone, Spain; Roche Products Limited, Welwyn, United Kingdom; Washington Cancer Institute, Washington Hospital Center, Washington, DC.

10:45  SS-6. Neoadjuvant pertuzumab and trastuzumab concurrent or sequential with an anthracycline-containing or concurrent with an anthracycline-free standard regimen: A randomized phase II study (TRYPHAENA)
Schneeweiss A, Chia S, Hickish T, Harvey V, Enull A, Hegg R, Tausch C, Seo J-H, Tsai Y-F, Ackrill A, Ross G, Cortés J. National Center for Tumor Diseases, University Hospital, Heidelberg, Germany; British Columbia Cancer Agency - Vancouver Centre, University of British Columbia, Vancouver, Canada, Royal Bournemouth Hospital, Bournemouth University, Bournemouth, United Kingdom; Auckland City Hospital, Auckland, New Zealand; Cancer Institute "I. Chitucita", Cluj-Napoca, Romania; Hospital Pêrola Byington, São Paulo, Brazil; Breast-Center, Zürich, Switzerland; Korea University Guro Hospital, Guro, Korea; Taipei Veterans General Hospital, Taiwan, Taiwan; Roche Products Limited, Welwyn, United Kingdom; Vall d’Hebron University Hospital, Barcelone, Spain.

11:00  SS-7. A phase 2, randomized, open-label, study of neratinib (HKI-272) vs lapatinib plus capecitabine for 2nd/3rd-line treatment of HER2+ locally advanced or metastatic breast cancer
Martin M, Bonnetterre J, Geyer, Jr CE, Ito Y, Ro J, Lang I, Kim S-B, Germa C, Vermette J, Vo Van ML, Wang K, Wang K, Awada A. Hospital Universitario Gregorio Marañon, Madrid, Spain; Centre Oscar Lambret, Lille, France; Allegheny Cancer Center, Pittsburgh, PA; The Cancer Institute Hospital of JFCR, Tokyo, Japan; National Cancer Center, Goyang, Korea; National Institute of Oncology, Budapest, Hungary; Asan Medical Center, Seoul, Korea; Pfizer Global Research and Development, Paris, France; Pfizer Inc, Collegeville, PA; Bristol Meyers Squibb, Princeton, NJ; Jules Bordet Institute, Brussels, Belgium.

11:15  Discussion
C. Kent Osborne, Baylor College of Medicine, Houston, TX.
11:30 am–12:00 pm

AACR OUTSTANDING INVESTIGATOR AWARD FOR BREAST CANCER RESEARCH, FUNDING BY SUSAN G. KOMEN FOR THE CURE*
Exhibit Hall D
Alteration and Inhibition of PTEN in Breast Cancer
Ramon E. Parsons, MD, PhD
Columbia University
New York, NY

12:00 pm–1:35 pm
LUNCH

12:15 pm–1:15 pm
PRODUCT THEATRE
Exhibit Hall C – Exhibit Area
Abrazane
Sponsored by Celgene Corporation

12:30 pm–1:35 pm
CASE DISCUSSION 2
Ballroom A
Moderator: Mothaffar Rimawi, MD
Baylor College of Medicine
Houston, TX
Panelists:
Kimberly Blackwell, MD
Duke University
Durham, NC
Jennifer Bellon, MD
Dana-Farber Cancer Institute
Boston, MA
Ismail Jatoi, MD
UT Health Science Center
San Antonio, TX
Martine Piccart, MD, PhD
Institut Jules Bordet
Brussels, BELGIUM
Vernal Branch
Virginia Breast Cancer Foundation
Richmond VA

12:30 pm–1:35 pm
BASIC SCIENCE FORUM
Ballroom B
Tumor Microenvironment
Moderator: Rong Li, PhD
UT Health Science Center San Antonio
San Antonio, TX
Extrinsic and Intrinsic Force Regulation of Breast Cancer Progression and Treatment
Valerie M. Weaver, PhD
University of California San Francisco
San Francisco, CA
COX-2 dependent collagen fibrillogenesis drives metastasis in the postpartum involuting mammary gland
Pepper J. Schedin, PhD
University of Colorado Denver
Aurora, CO

1:45 pm–3:15 pm

MINI-SYMPOSIUM 3
Ballroom B
Metabolism in Breast Cancer - Metabolomics
Moderator: Michael Pollak, MD
McGill University
Montreal, CANADA
Adiposity, metabolism, and risk of breast cancer
Susan E. Hankinson, ScD
Harvard Medical School
Boston, MA
HIF-1, metabolism, and metastasis
Gregg L. Semenza, MD, PhD
The Johns Hopkins University School of Medicine
Baltimore, MD
Energy metabolism in breast cancer: translational science insights relevant to effects of diet, exercise, and metformin on risk and prognosis
Michael Pollak, MD
McGill University
Montreal, CANADA

1:45 pm–3:15 pm
MINI-SYMPOSIUM 4
Exhibit Hall D
The Role and Promise of Neoadjuvant Therapy in Breast Cancer
Moderator: Lisa A. Carey, MD, ScM
University of North Carolina
Chapel Hill, NC
The neoadjuvant setting, HER2-driven and triple negative breast cancer
Lisa A. Carey, MD, ScM
University of North Carolina
Chapel Hill, NC
Preoperative endocrine therapy: New approaches
Ian Smith, MD
Royal Marsden Hospital
London, UNITED KINGDOM
Interface with radiation oncology
Thomas A. Buchholz, MD
UT MD Anderson Cancer Center
Houston, TX
Neoadjuvant trials as a discovery tool
Carlos L. Arteaga, MD
Vanderbilt-Ingram Cancer Center
Nashville, TN

3:15 pm–5:00 pm
GENERAL SESSION 6
Exhibit Hall D
Moderator: Pamela Goodwin, MD
Mount Sinai Hospital
New York, NY
Vernal Branch
Virginia Breast Cancer Foundation
Richmond VA

Extrinsic and Intrinsic Force Regulation of Breast Cancer Progression and Treatment
Valerie M. Weaver, PhD
University of California San Francisco
San Francisco, CA
COX-2 dependent collagen fibrillogenesis drives metastasis in the postpartum involuting mammary gland
Pepper J. Schedin, PhD
University of Colorado Denver
Aurora, CO

12:00 pm–1:35 pm

LUNCH

12:15 pm–1:15 pm
PRODUCT THEATRE
Exhibit Hall C – Exhibit Area
Abrazane
Sponsored by Celgene Corporation

12:30 pm–1:35 pm
CASE DISCUSSION 2
Ballroom A
Moderator: Mothaffar Rimawi, MD
Baylor College of Medicine
Houston, TX
Panelists:
Kimberly Blackwell, MD
Duke University
Durham, NC
Jennifer Bellon, MD
Dana-Farber Cancer Institute
Boston, MA
Ismail Jatoi, MD
UT Health Science Center
San Antonio, TX
Martine Piccart, MD, PhD
Institut Jules Bordet
Brussels, BELGIUM
Vernal Branch
Virginia Breast Cancer Foundation
Richmond VA

12:30 pm–1:35 pm
BASIC SCIENCE FORUM
Ballroom B
Tumor Microenvironment
Moderator: Rong Li, PhD
UT Health Science Center San Antonio
San Antonio, TX
Extrinsic and Intrinsic Force Regulation of Breast Cancer Progression and Treatment
Valerie M. Weaver, PhD
University of California San Francisco
San Francisco, CA
COX-2 dependent collagen fibrillogenesis drives metastasis in the postpartum involuting mammary gland
Pepper J. Schedin, PhD
University of Colorado Denver
Aurora, CO

1:45 pm–3:15 pm
MINI-SYMPOSIUM 3
Ballroom B
Metabolism in Breast Cancer - Metabolomics
Moderator: Michael Pollak, MD
McGill University
Montreal, CANADA
Adiposity, metabolism, and risk of breast cancer
Susan E. Hankinson, ScD
Harvard Medical School
Boston, MA
HIF-1, metabolism, and metastasis
Gregg L. Semenza, MD, PhD
The Johns Hopkins University School of Medicine
Baltimore, MD
Energy metabolism in breast cancer: translational science insights relevant to effects of diet, exercise, and metformin on risk and prognosis
Michael Pollak, MD
McGill University
Montreal, CANADA

1:45 pm–3:15 pm
MINI-SYMPOSIUM 4
Exhibit Hall D
The Role and Promise of Neoadjuvant Therapy in Breast Cancer
Moderator: Lisa A. Carey, MD, ScM
University of North Carolina
Chapel Hill, NC
The neoadjuvant setting, HER2-driven and triple negative breast cancer
Lisa A. Carey, MD, ScM
University of North Carolina
Chapel Hill, NC
Preoperative endocrine therapy: New approaches
Ian Smith, MD
Royal Marsden Hospital
London, UNITED KINGDOM
Interface with radiation oncology
Thomas A. Buchholz, MD
UT MD Anderson Cancer Center
Houston, TX
Neoadjuvant trials as a discovery tool
Carlos L. Arteaga, MD
Vanderbilt-Ingram Cancer Center
Nashville, TN

3:15 pm–5:00 pm
GENERAL SESSION 6
Exhibit Hall D
Moderator: Pamela Goodwin, MD
Mount Sinai Hospital
New York, NY
Vernal Branch
Virginia Breast Cancer Foundation
Richmond VA

Extrinsic and Intrinsic Force Regulation of Breast Cancer Progression and Treatment
Valerie M. Weaver, PhD
University of California San Francisco
San Francisco, CA
COX-2 dependent collagen fibrillogenesis drives metastasis in the postpartum involuting mammary gland
Pepper J. Schedin, PhD
University of Colorado Denver
Aurora, CO
3:15  **56-1.** Menopause-specific and health-related qualities of life among post-menopausal women taking exemestane for prevention of breast cancer: Results from the NCIC CTG MAP.3 placebo-controlled randomized controlled trial
Maursell E, Richardson H, Ingle JN, Ales-Martinez JE, Chlebowski RT, Fabian CJ, Sarto GE, Garber JE, Pujoel P, Hiltz A, Tu D, Goss PE. Université Laval, Quebec City, QC, Canada; Queen’s University, Kingston, ON, Canada; Mayo Clinic, Rochester, MN; Hospital Ntra Sra Sonsoles, Avila, Spain; Los Angeles Biomedical Research Institute, Torrance, CA; University of Kansas Medical Center, Westwood, KS; Center for Women’s Health and Health Research, Madison, WI; Dana Farber Cancer Institute, Boston, MA; CHU-Hopital Arnaud de Villeneuve, Montpellier, France; Massachusetts General Hospital Cancer Center, Boston, MA.

3:30  **56-2.** Patient-reported predictors of early treatment discontinuation: NCIC JMA.27/E1203 quality of life study of postmenopausal women with primary breast cancer randomized to exemestane or anastrozole
Wagner LL, Zhao F, Chapman J-AW, Cella D, Shepherd LE, Sleight GW, Goss PE. Northwestern University Feinberg School of Medicine, Chicago, IL; Dana-Farber Cancer Institute, Boston, MA; NCIC Clinical Trials Group, Queen’s University, Kingston, ON, Canada; Indiana University Simon Cancer Center, Indianapolis, IN; Massachusetts General Hospital, Boston, MA.

3:45  **Discussion**
Patricia A. Ganz, MD
UCLA Jonsson Comprehensive Cancer Center
Los Angeles, CA

4:00  **56-3.** Influence of hospital factors, physician factors and type of health insurance on receipt of immediate postmastectomy reconstruction in young women with breast cancer
Hershman DL, Neugut AI, Richards CA, Kalinsky K, Charles AS, Wright JD. Columbia University, New York, NY; Mailman School of Public Health, New York, NY.

4:15  **56-4.** Protein kinase mutation patterns in human breast cancer

4:30  **56-5.** Obesity drives epithelial-to-mesenchymal transition and tumor progression in a novel claudin-low mammary cancer model
Dunlap SM, Chiao LJ, Nurieua L, Usary J, Perou CM, Varticovski L, Hurston SD. University of Texas, Austin, TX; University of North Carolina, Chapel Hill, NC; National Cancer Institute, Bethesda, MD; University of Texas M.D. Anderson Cancer Center, Smithville, TX.

4:45  **56-6.** Expression of key estrogen-regulated genes differ substantially across the menstrual cycle in ER+ breast tumours
Haynes BP, Viale B, A-Hemi R, Smith IE, Dowsett M, Galimberti V, Rotmensz N, Gibe1li B. Royal Marsden Hospital, London, United Kingdom; Institute of Cancer Research, Sutton, Surrey, United Kingdom; European Institute of Oncology, Milan, Italy.

5:00 pm–7:00 pm
**POSTER SESSION 5 & RECEPTION**
Exhibit Halls A-B

**Tumor Cell Biology: Biomarkers**

**PS-01-01 Identification, Validation and Assessment of Transcriptional Relevance of a PDGFR-Activation Signature in (Inflammatory) Breast Cancer**
Van Laere SJ, Van Golen KL, Joglekar M, Ueno NT, Finetti P, Van Dam PA, Viens P, Bimbbaum D, Bertucci F, Vermeulen PB, Dirix LY. Oncology Center - GH Sint-Augustinus, Wilrijk, Belgium; University of Delaware, Newark, DE; The University of Texas MD Anderson Cancer Center, Houston, TX; Institut Paoli-Calmettes (IPC), Marseille, France; World IBC Consortium; Contributed Equally.

**PS-01-02 Wnt5a Is a Prognostic Biomarker in Estrogen Receptor-Positive Premenopausal Breast Cancer**
Sand-Jensen JT, Ehrenstrom R, Andersson T, Ryden L. Clinical Sciences, Skåne University Hospital, Lund, University, Malmö, Sweden; Laboratory Medicine, Skåne University Hospital, Lund University, Malmö, Sweden.

**PS-01-03 Correlation between Aromatase Expression in Metastatic and Primary Breast Cancer**
Ribeiro JM, Luis IV, Correia L, Casimiro S, Fernandes A, Quintela A, Mestan J, Ramos M, Costa L. Hospital de Santa Maria, Lisbon, Portugal; University of Lisbon, Lisbon; Hospital de Santa Maria, Lisbon; Novartis Pharma.

**PS-01-04 FOXP3 Positive Regulatory T Lymphocytes and Epithelial FOXP3 Expression in Synchronous Normal, Ductal Carcinoma In Situ and Invasive Cancer of the Breast**

**PS-01-05 Activating Mutations in PIK3CA or AKT1 in the I-SPY 1 Trial (CALGB 150007/150012; ACRIN 6657)**
Boudreau A, You C, Petrolli L, Stemke-Hale K, Mills GB, Gray J, Wolf DM, van’t Veer LJ, The I-SPY 1 TRIAL Investigators, Esserman LJ, University of California, San Francisco; University of Texas MD Anderson Cancer Center; Oregon Health & Science University.

**PS-01-06 Gene Copy Number and Expression of TYMP and TMS Are Predictive of Outcome in Breast Cancer Patients Treated with Capecitabine**
Audet RM, Changyu S, Duchnowska R, Adamowicz K, Zok J, Rogowski W, Litwinuk M, Desbska S, Jawonska M, Foszczyńska-Klocla M, Kulma-Kreft M, Zabkowska K, Jassem J, Edgerton S, Yang Nielsen K, Thor A, Chang J, Miller K, Sledge GW. Yale University of Research, Montreal, QC, Canada; Medical University of Gdańska, Gdańska, Poland; Indiana University School of Medicine, Indianapolis, IN; Melvin and Ben Simon Cancer Center, Indianapolis; Emory University, Atlanta, GA; UC Denver School of Medicine, Aurora, CO; DAKO, Glostrup, Denmark; The Methodist Hospital Research Institute, Houston, TX; Military Institute of Medicine, Warsaw, Regional Oncology Center, Olsztyn; Poznan University of Medical Sciences; Regional Cancer Center, Lodz, MD; District Hospital, Wrocław, MD; West Pomeranian Oncology Center, Szczecin; Gdynia Oncology Center.

**PS-01-07 Identification of SORBS2 as a Candidate Marker To Predict Metastatic Relapse in Breast Cancer**

**PS-01-08 Immunocytchemistry Staining of Estrogen Receptor in Circulating Tumor Cells as Compared to Primary Tumor**
Mayer JA, Pham T, Wong KL, Birscholf FZ. Biocent Inc, San Diego, CA.

**PS-01-09 Differences in Serum Estradiol and Prolactin Concentrations in Women Who Yield Nipple Aspiration Fluid and Those Who Do Not**
Khan SA, Fought AJ, Scholten DM, McGathey C, Hintz RE, Chatterton RT. Northwestern University, Feinberg School of Medicine, Chicago, IL.

**PS-01-10 A Potential New Marker for, and Facilitator of, Hormone Independence**
Monaco ME, Wu X, Lee P. NYU School of Medicine, New York, NY; VA NY Harbor Healthcare System, New York, NY.

**PS-01-11 Small Node-Negative (T1b-C0) Invasive Hormone Receptor (HR)-Positive Breast Cancers: Is There a Population Which Might Have Benefit from Adjuvant Chemotherapy?**
Cancer Res; 71(24 Suppl.) December 15, 2011

PS-05-03 The Effect of Breastfeeding on Molecular Characteristics of Invasive Breast Cancer
Ellsworth RE, Valente AL, Kane JL, Shriver CD, Henry M. Jackson Foundation, Windber, PA; Windber Research Institute, Windber, PA; Walter Reed Army Medical Center, Washington, DC.

PS-05-04 Tamoxifen-Induced Apoptosis Is Compromised by Bisphenol-A and Methylparaben Exposure
Goodson III WH, Luciani MG, Sayed A, Jaffe J, Moore II DH, Dairkee SH. California Pacific Medical Center Research Institute, San Francisco, CA; Kimmel Cancer Center, San Francisco, CA; California Pacific Medical Center, San Francisco, CA; University of California, San Francisco, CA.

PS-05-05 Human Papilloma Virus Identification in Intraductal Papilloma and Breast Cancer Using Broad-Spectrum Primers
Balić FL, Bender O, Coskun F, Duzgun AP, Saylam B, Yuney E, Rota S, Fidan I, Feldman S, Soran A. Numune Training and Research Hospital, Ankara, Turkey; Okmeydani Training and Research Hospital, Istanbul, Turkey; Gazi University School of Medicine, Turkey; Columbia University College of Physicians and Surgeons, New York; University of Pittsburgh, Magee-Womens Hospital, Pittsburgh.

PS-05-06 Dysregulation in the Transport of Iron and Haem Is Implicated in the Aetiopathogenesis of Breast Cancer
Roe T, Hoar F, Tslepis C. University of Birmingham, Birmingham, United Kingdom; City Hospital, Birmingham, United Kingdom.

PS-05-07 The Msp1 Polymorphism of Cytochrome P-450 CYP1A1 in Asymptomatic Women with Breast Cysts
Fensile R, Nazario A, Facina G. Federal University of Sao Paulo, Sao Paulo, SP, Brazil.

Tumor Cell Biology: Drug Resistance

PS-06-01 Gene Expression Analysis of Resistance to Bevacizumab in a VEGF-Reinforced Xenograft Model of ER-Positive Breast Cancer
Golken-Polar Y, Toroni RA, Goswami C, Sanders KL, Mehta R, Sinmalle U, Tansaa B, Chen C, Li I, Ivan M, Badve S, Sledge GW. Indiana University School of Medicine, Indianapolis, IN; University of Medicine and Pharmac, La Jolla, CA.

PS-06-02 Response and Acquired Resistance of BRCA1-Deficient Triplet-Negative Breast Cancer Xenografts to Alkylators or PARP Inhibitors

PS-06-03 Gene Expression Associated with Breast Cancer Primary and Secondary Resistance to Neoadjuvant Chemotherapy
Fujita FK, Katayama MLH, Brentani H, Carraro DM, Abeu APS, Barros Filho MC, Oliveira CT, Caldeira JRF, Góes JCS, Brentani MM, Folgueira MAX. Faculdade de Medicina da Universidade de Sao Paulo, Sao Paulo, Brazil; Faculdade de Medicina da USP, Sao Paulo, Brazil; Hospital do Cancer A.C. Camargo, Sao Paulo, Brazil; Instituto Brasileiro de Controle do Cancer, Sao Paulo, Brazil; Hospital do Cancer A.C. Camargo, Sao Paulo, Brazil; Hospital Amaral Carvalho, Jau, Sao Paulo, Brazil.

PS-06-04 Mechanisms of Acquired Resistance to Insulin-Like Growth Factor 1 Receptor Inhibitor in MCF-7 Breast Cancer Cell Line
Ekyalongo RC, Mukohara T, Kataoka Y, Kyota N, Fujiwara Y, Minami H. Kobe University Graduate School of Medicine, Kobe, Japan; Kobe University Hospital Cancer Center, Kobe, Japan.

PS-06-05 Withdrawn

PS-06-06 The RNA Binding Protein FUS Is a Potential Marker for Breast Cancer Progression and Therapy Response
Brooke GN, Bevan CL, Rudraraju B, Palmieri C. Imperial College London, London, United Kingdom.

Tumor Cell Biology: Breast Cancer Xenografts


PS-06-08 Mesenchymal Stem Cells and Carcinoma-Associated Fibroblasts Sensitize Breast Cancer Cells in 3D Cultures to Kinase Inhibitors
Dittmer J, Dittmer A, Oerlecke J, Leyh B, Martens JMV, Thomssen C. Clinic for Gynecology University Halle, Germany; Erasmus Medical Center.

PS-06-09 Acquired Sensitivity to TRAIL Mediated Apoptosis in Lapatinib Resistant SKBR3 Cells
Eustace AJ, Browne BC, McDermott M, Gallagher C, Watson W, Crown J, O’Donovan N. Dublin City University, Dublin, Ireland; University College Dublin, Dublin, Ireland; St. Vincents University Hospital, Dublin, Ireland.

PS-06-10 Leptin Signaling Impacts Notch and Wnt Crosstalk in Breast Cancer
McGlothen TZ, Gillaume C, Colbert L, Blaylock-Hogans D. Morehouse School of Medicine, Atlanta, GA; Spelman College, Atlanta, GA.

PS-06-11 Quercetin-3-Methyl Ether Inhibits Lapatinib-Sensitive and Lapatinib-Resistant Breast Cancer Cell Growth by Inducing G2/M Arrest and Apoptosis
Bode AM, Li J, Zhu F, Normanno NE, Ericson ME, Lubeet GA. University of Minnesota, Austin, MN; INT-Fondazione Pascale, Naples, Italy; University of Minnesota, Minneapolis, MN; National Cancer Institute, Rockville, MD.

Tumor Cell Biology: Breast Cancer Xenografts

PS-07-01 Novel Alternative Splice Variants of HER2 in Invasive Breast Cancer
Assam EE, Rhodes A, Ladomery M, Harries L, Sohal M. University of the West of England, Bristol, Gloucestershire, United Kingdom; Royal Devon and Exeter NHS Foundation Trust Hospital, Exeter, Devon, United Kingdom; United Hospitals Bristol Foundation Trust, Bristol, Gloucestershire, United Kingdom.

PS-07-02 Could the Combination of COX-2 Inhibitor and Calcitriol Be a New Chemopreventive Approach To Decrease the Incidence of Breast Cancer?

PS-07-03 Modulation of Autophagic Activity by Extracellular pH
Xu T, Su H, Ganapathy S, Yuan Z-M. University of Texas Health Science Center at San Antonio, San Antonio, TX.

PS-07-04 Is α-L-Fucose Overexpressed on Cells of Aggressive Human Breast Cancers?
Listinsky JJ, Siegal GP, Listinsky CM. University of Alabama at Birmingham, Birmingham, AL; Case Western Reserve University, Cleveland, OH.

PS-07-05 Hypoxic Mammary Epithelial Cells in Three-Dimensional Culture Develop a Cancer-Like Phenotype
Vaapil M, Helcynska K, Pählinan S, Jögi A. Lund University, Sweden.

PS-07-06 Effect of Angiotensin-(1-7) and Angiotensin II on T47D Breast Cancer Cells in the Proliferation and cAMP Production
Correa-Noronha SSAA, Noronha SMR, Alercim C, Shimuta SI, Nakae CR, Gebrim LH, Nazario ACP, Silva IDCG. UNIFESP, Sao Paulo, SP, Brazil.

PS-07-07 Follistatin Suppresses In Vitro Growth of Breast Cancer Cells and Its Reduced Expression in Breast Cancer Associated with Poor Differentiation and Prognosis
Ye L, Mansel RE, Jiang WG. Cardiff University School of Medicine, Cardiff, United Kingdom.

Cancer Res; 71(24 Suppl.) December 15, 2011

68s Cancer Research
PS-07-08  Pomegranate extract targets miRNA-27a and miRNA-155 in the reduction of inflammation and cell growth in ER+ breast cancer in vitro and vivo  
Mertens-Talcott SU, Banerjee N, Safe S, Talcott S. Texas A&M University, College Station, Texas; Texas A&M Health Science Center, College Station, Texas.

Detection and Diagnosis – Imaging and Screening: Breast Imaging – Mammography

PS-08-01  Quantitative Analysis of Strain Ratio of Microcalcification Lesions with Surgical Specimen Mammography  
Hung S-H, Chen R-C, Wu Y-T, Chen T, Lin C-H. Taipei City Hospital, Taiwan; National Yang- Ming University, Taiwan.

PS-08-02  Mammographic Breast Texture Predicts Benign Biopsy Results and Composition  

PS-08-03  How Reader’s Training, Software, and Image Formats Impact Percent Dense Area Measures  
Fan B, Duever F, Wu F, Kertikowske K, Vachon C, Shepherd JA. University of California San Francisco, San Francisco, CA; Mayo Clinic College of Medicine, Rochester, MN.

PS-08-04  Mammographic Microcalcifications and Breast Cancer Tumorigenesis: A Radiologic-Pathologic Analysis  

PS-08-05    Withdrawn

PS-08-06  Comparison of Mammographic Density between Ductal Carcinoma In Situ and Benign Breast Disease  

Detection and Diagnosis – Imaging and Screening: Screening

PS-09-01  Breast Cancer Annual Screening Program Núcleo Mama Porto Alegre (NMPOA): Imaging Results after Seven Years  

PS-09-02  Reducing Excess Biopsies: Improving Screening through Risk Stratification and New Thresholds for Intervention  
Kim DN, Kim J-H, Flowers CJ, Elias S, Moore DH, Esserman LJ. Athena Breast Health Network, San Francisco, CA; Moffitt Cancer Center, Tampa, FL; University Medical Center Utrecht, Utrecht, Netherlands; University of California, San Francisco, CA.

PS-09-03  Health Literacy Affects Use of Screening Mammography in an Underinsured Population  
Komenaka IK, Nodora J, Hsu C-H, Machado L, Klemens AE, Zenuk R, Bouton ME, Martinez ME, Weiss BD. Maricopa Medical Center, Phoenix, AZ; University of Arizona, Tucson, AZ.

PS-09-04  Predictors of Malignancy and Surgical Outcomes Following Indeterminate Core Needle Biopsy in the British Breast Screening Programme  
Gillespie HS, Lowry K, Somerville J, McIntosh SA. University of Aberdeen, Foresterhill, Aberdeen, Aberdeenshire, Scotland, United Kingdom; Belfast City Hospital, Belfast, County Down, United Kingdom.

PS-09-05  Non-Palpable Lesions Findings in a Breast Cancer Opportunistic Screening in South Brazil  

PS-09-06  Breast cancer detection through metabolic fingerprint in blood  

Detection and Diagnosis: Imaging and Screening: Radiology – Tumor Monitoring

PS-10-01  Metabolic Pharmacodynamic Effect Evidenced by 18FDG-PET as a Tool for Early Prediction of Iniparib Efficacy in Metastatic Triple Negative Breast Cancer (TNBC): A Proof of Concept Study  
Kerrou K, Gligorov J. Eastern Paris University Hospitals, APHP Tenon, Paris, France; Eastern Paris University Hospitals, APHP Tenon - APREC, Paris, France.

PS-10-02  Relevance of Magnetic Resonance Imaging To Predict Disease-Free Survival after Neoadjuvant Chemotherapy of Breast Cancer  
Loo CE, Vrancken Peeters M-JTFD, Wesseling J, Gilhuys KG. NKI-AVL (Netherlands Cancer Institute - Antoni van Leeuwenhoek Hospital), Amsterdam, Netherlands; University Medical Centre Utrecht, Utrecht, Netherlands; NKI-AVL, Amsterdam, Netherlands.

Detection and Diagnosis – Pathology: Diagnostic Pathology

PS-11-01  The Accuracy of Preoperative Ultrasonography Guided Vacuum-Assisted Breast Biopsy in Determining Histological Type, ER Status, PgR Status, HER2 Status and Ki67 Level in Invasive Breast Cancer  

PS-11-02  Prediction of Results of MammaPrint’s 70 Gene Signatures by Conventional Histopathological and Biological Approaches in Patients with Breast Cancer  

PS-11-03  Real-Time Imaging of Human Breast Tissue with Reflectance Confocal Microscopy: Correlation with Routine Pathology  
Fogarty SP, Shiffert MT, Berezowski K, Hartmann D, Cabrera MC, Sidaway MK, Furth PA, Liu MC. Georgetown University, Washington, DC.

PS-11-04    Withdrawn

PS-11-05  Measurement of Neoadjuvant Chemotherapy Tumor Response in Locally Advanced Breast Cancer by Three Methodologies. Correlation with Overall Survival  
Garcia-Saenz JA, Romero A, Lopez Garcia-Asenjo JA, Roman JM, Moreno A, Fuentes M, Furto V, Pelayo A, Diaz-Rubio E, Caldes T, Martin M. Hospital Clinico San Carlos, Madrid, Spain; Hospital Principe de Asturias, Alcala de Henares, Madrid, Spain; Hospital Gregorio Maranon, Madrid, Spain.

PS-11-06  Immunohistochemistry Discordance between Primary and Recurrent Tumors in Breast Cancer. Analysis of Potential Influence of Technique Bias by Comparing Test-Results under Two Different Conditions  

PS-11-07  Receptor Concordance in Triple-Negative Breast Cancer (TNBC) Recurrences  
Killian ME, De Los Santos JF, Foreno-Torres A, Krontiras H. University of Alabama at Birmingham, Birmingham, AL.
PS-11-08  High Concordance of 5 HER2 In Situ Hybridization Methods with Abbott FISH  
Boers JE, Netjes C, Meeuwissen HC, Prinsen C, Bart J, van der Logt EMJ, Schuuring E, Isala Klinieken, Zwolle, Netherlands; University Medical Center, Groningen, Netherlands.

PS-11-09  High Concordance for Microarray Based Determination of ER, PR and HER2 Receptor Status and Local IHC/FISH Assessment Worldwide in 749 Patients  
Wesseling J, Cusumano G, Tintemari C, Sapino A, Zanconati F, Fukutake-Holzlik N, Nguyen B, Deck K, Querzoli P, Penn T, Giardina C, Seitz G, Guinebretiere J, Barone J, Watanabe T. Netherlands Cancer Institute, Amsterdam, Netherlands; CHC, Liege, Belgium; Instituto Clinico Humanitas, IRCCS, Rozzano, Italy; Universita di Torino, Torino, Italy; Universita di Trieste, Trieste, Italy; Medisch Spectrum Twente, Enschede, Netherlands; Locg Beach Memorial Health Care, Loch Beach, CA; Saddleback Memorial Medical Center, Laguna Hills, CA; Instituto di Patologia, Universita di Ferrara, Ferrara, Italy; Centro di Riferimento Oncologico, Aviano, Italy; Instituto di Anatomia Patologica, Universita degli Studi di Bari, Bari, Italy; Klinikum Bamberg, Bamberg, Germany; Centre Rene Huguenin, Saint-Cloud, France; Comprehensive Breast Care and Sharp Memorial Hospital, San Diego, CA; Hamamatsu Oncology Center, Hamamatsu, Japan.

PS-11-10  Reproducibility and Robustness of the FDA Approved INFORM HER2 Dual ISH DNA Probe Cocktail Assay  

PS-11-11  Automated Quantification Methods Improve the Accuracy of PR as an Independent Prognostic Factor in Tamoxifen Treated Breast Cancer Patients  
Klimowicz AC, Komaga EN, Yau A, Pohorelic BK, Petillo SK, Konno M, Magliocco AM. Tom Baker Cancer Centre, Calgary, AB, Canada; University of Calgary, Calgary, AB, Canada.

PS-11-12  Correlation of Ki67 Expression between Initial Biopsy and Surgical Specimen in Untreated Breast Cancer Patients: Does Menstrual Cycle Matter?  

PS-11-13  Rates of Upgrade to Malignancy for 271 Cases of Atypical Columnar Cell Hyperplasia Diagnosed by Breast Core Biopsy  
Peres A, Barranger E, Becette V, Boudinet A, Guinebretiere J-M, Chere P. Lariboisiere Hospital, Paris, France; Institut Curie, Saint-Cloud, France.

PS-11-14  Flat Epithelial Atypia of the Breast: A Single Institution Experience  

PS-11-15  Should HER-2 Score 0/1+ Breast Cancer Cases Be Retested by In-Situ Hybridisation? Results of a Multicenter Retesting Study  
Reiner-Concin AM, Lack S, Regina-Toni P, Kronberger C, Jasarevic Z, Bogner S. Danube Hospital, Vienna, Austria; General Hospital Graz West, Graz, Austria; Medical University Graz, Graz, Austria; LHU Salzburg, Salzburg, Austria; LHK Feldkirch, Feldkirch, Austria; LKH Linz, Linz, Austria.

PS-11-16  CD44 and CD24 Expression in Ductal Invasive Breast Carcinomas, Classified by Molecular Subtypes and Its Association with Prognostic Factors  
Bernardi MA, Logullo AF, Pasini F, Nonogaki S, Soares FA, Maciel MD, Brentani MM. Hospital AC Camargo, Sao Paulo, Brazil; Universidade Federal de Sao Paulo, Sao Paulo, Brazil; Faculdade de Medicina da Universidade de Sao Paulo, Sao Paulo, Brazil; Hospital A.C. Camargo, Sao Paulo, Brazil.

PS-11-17  Evaluating Tumor Heterogeneity in Immunohistochemistry Stained Breast Cancer Tissue  
Portis SJ, Landis N, Eberhard DA, Schmehel SC, Young D, Lange H. Flagship Biosciences, Flagstaff, AZ; University of North Carolina, Chapel Hill, NC; University of Minnesota, Minneapolis, MN.

Detection and Diagnosis – Pathology: Detection and Diagnosis – Other  
PS-12-01  Aromatase Inhibitor Specific Metastasis Is Driven by the Steroid Receptor Coactivator SRC-1  
Thiesen SM, McBayan J, Byrne C, Hughes E, Coccigia S, Hill AD, Young LS. Royal College of Surgeons in Ireland, Dublin 2, Ireland.

PS-12-02  Vacuum Assisted Biopsies of Ductal Carcinoma In Situ and Concordance with Post-Operative Histology: Implications for the Low Risk DCIS Trial  
Soumian S, Down SK, Roked F, Chaudhri S, Francis A. Queen Elizabeth Hospital, University Hospitals Birmingham, Birmingham, England, United Kingdom.

PS-12-03  An Innovative Quantification Method for Tamoxifen and Three Metabolites in Formalin-Fixed Paraffin-Embedded Tissues by Liquid Chromatography and Tandem Mass Spectrometry  
Magliocco AM, Ng ES, Kangaroo B, Konno M, Paterson A. Tom Baker Cancer Centre, Calgary, AB, Canada; University of Calgary, Calgary, AB, Canada.

PS-12-04  Genetic Linkage between Acquired and Primary Lymphedema Evaluated through Whole Exome Sequencing and NIR Fluorescence Lymphatic Imaging  
Sevick-Muraca EM, Gonzalez-Garay ML, Fife CE, Guilliod R, Hall O, Marshall MV, Rasmussen JC, Aldrich MB, Darne C, Zhu B, Tan I-C, Caskey CT. University of Texas Health Science Center, Houston, TX; Memorial Hermann Hospital, Houston, TX.

PS-12-05  (In-)Efficiencies in the Preoperative Imaging Evaluation of the Medicare Breast Cancer Patient  

PS-12-06  Gastrointestinal metastasis of invasive lobular breast carcinoma: Brazilian Cancer Institute review from March 2004 to April 2009  

PS-12-07  Physicians’ call to use mammary ductoscopy in patients without spontaneous nipple discharge  
Xu Z, Dou T-H, Wu S, Xing H, Song C, Dou C. Norman Bethune Medical School, LiU University, Changchun, Jilin Province, China; Dehui County Hospital, Dehui, Jilin Province, China; Indiana University, Indianapolis, IN; Georgia University System/Georgia Gwinnett College, Lawrenceville, GA.

Prognosis/Response Predictions: Response Predictions – II  
PS-13-01  Survival Outcome with Bevacizumab: Activation of the Phosphatidylinositol-3 Kinase (PI3K) Pathway Due to PI3KCA Mutations or PTEN Loss Makes a Difference  

PS-13-02  Prediction of Dasatinib Sensitivity of Breast Cancer Based on a Novel Tyrosine Kinase-Activity Profiling Assay  
Kawai M, Tonkoshi Y, Notoya M, Gohda K, Ueno NT, Ishihara H. Sysmex Corporation, Kobe, Hyogo, Japan; The University of Texas MD Anderson Cancer Center, Houston, TX.
Discordance of Estrogen Receptor and HER-2/neu Status Can Be Seen between First and Subsequently Biopsied Metastatic Lesions in Breast Cancer
Lower EE, Kennedy D. Oncology Hematology Care, Cincinnati, OH.

PTEN and Tau-Protein Expression: Predictive Value of Poor Response to Trastuzumab Plus Paclitaxel in Patients with HER2-Positive Breast Cancer
Koo DH, Ahn J-H, Youn DH, Kim S-B, Lee HJ, Kong KY, Son BH, Ahn SH, Jung KH. Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea.

Can the Ki67 Proliferation Index Predict the Oncotype DX Recurrence Score in Lymph Node Negative, ER Positive Breast Cancer?
Prendergast A, Martin K, Doolan P, Clarke C, Clynes M, Eddy D, McDermott E, Crawn J, Kennedy S. Dublin City University, Dublin, Ireland; Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Dublin, Ireland; St. Vincent’s University Hospital, Dublin, Ireland.

The Role of Topoisomerase IIα in Predicting Sensitivity to Anthracyclines in Breast Cancer Patients: A Meta-Analysis of Published Literatures
Lu L, Du Y, Zou Q, Yin W, Zhou L, Di G, Shen Z, Shao Z. Shanghai Cancer Center, Fudan University, Shanghai, China; Shanghai Medical College, Fudan University, Shanghai, China.

Drug-Metabolizing Enzyme Polymorphisms and Clinical Outcome of Anthracycline-Based Chemotherapy in Chinese Han Breast Cancer Patients
Tang J, Zhao J, Wu J, Ji M, Zhong S. Jiangsu Cancer Hospital, Nanjing, Jiangsu, China.

Proteomic Identification of Predictive Biomarkers of Resistance to Neoadjuvant Chemotherapy in Luminal Breast Cancer: A Possible Role for 14-3-3 and BID?
Hodgkinson VC, EL-Fadl D, Agarwal V, Garimella V, Drew PJ, Lind MJ, Cawkwell L. University of Hull, Hull, United Kingdom; Castle Hill Hospital, Hull, United Kingdom; Hull York Medical School, Hull, United Kingdom.

Reduction in PET Uptake-Value Is an Early Predictor for Response to Neoadjuvant Therapy Including Anthracycline and Taxane in Stage II-IIIA Breast

Multigene Signature Assays in Patients with Early-Stage Breast Cancer (ESBC) Receiving Neoadjuvant Chemotherapy: An NCI-Funded Systematic Review and Evidence Summary of Predictive Performance
Lyman GH, Cukalova E, Pieperwierski MS, Huang M, Barry W, Ginski G, Abemethy A, Marcom PK, Ready N, Kuderer NM. Duke University School of Medicine, Durham, NC; Duke University, Durham, NC.

A Study of the Usefulness of Tumor Markers CA 15-3 and TPS in Monitoring of Different Subgroups of Metastatic Breast Cancer

Borderline Estrogen and Progestosterone Receptor Expression and Efficacy of Anti-Estrogen Therapy Analyzed by Subpopulation Treatment Effect Pattern Plot Analysis
Luch S-W, Ramsey B, Park B, Keen A. Oregon Health and Science University, Portland, OR; Portland Veterans Administration Medical Center, Portland, OR.
PS-13-20  TOP2A Amplification Have Associated with Response to Anthracycline-Based Preoperative Chemotherapy in Primary Breast Cancer
Wang J, Xu B, Yuan P, Zhang P, Li Q, Ma F, Zhao L. Cancer Hospital, Chinese Academy of Medical Science, Beijing, China.

PS-13-21  Japanese Patients with Discordance in Estrogen Receptor between Primary Breast Cancer and Recurrent Tumor Have a Poorer Outcome

PS-13-22  Gene Expression Profiles Predict Pathological Complete Response to Standard Neoadjuvant Fluorouracil, Doxorubicin, and Cyclophosphamide and Paclitaxel with or without Trastuzumab in Early Breast Cancer
Tamura K. National Cancer Center Hospital, Tokyo, Japan.

PS-13-23  Individualized Treatment Strategies for HER2-Negative Breast Cancer Subtypes
Ishikawa T, Shimizu D, Yamada A, Sasaki T, Monita S, Tanabe M, Kawachi K, Nozawa A, Chishima T, Kimura M, Ichikawa Y, Endo I. Yokohama City University Medical Center, Yokohama, Kanagawa, Japan; Yokohama City University, Yokohama, Kanagawa, Japan.

PS-13-24  A Predictive Model of Early Systemic Disease Relapse after Standard Adjuvant Therapy for Breast Cancer

Prognosis/Response Predictions: Prognostic and Predictive Factors – Other

PS-14-01  Differences in Efficacy by Assessment Method: NCIC CTG Adjuvant Breast Cancer Trials MA.5, MA.12, MA.14, MA.21, MA.27 Meta-Analysis
Dong B, Chapman J-AW, Yerushalmi R, Goss PE, Pollak MN, Burnell MJ, Bramwell VH, Levine MN, Pritchard KI, Whelan T, Ingle JN, Parulekar W, Shepherd LE, Gelmon KA. NCIC Clinical Trials Group, Queen’s University, Kingston, ON, Canada; Vancouver Cancer Centre - BCNA; Vancouver, BC, Canada; Harvard Medical School, Boston, MA; McGill University, Montreal, QC, Canada; Atlantic Health Sciences Corporation, Saint John, NB, Canada; Alberta Cancer Board, Calgary, AB, Canada; McMaster University, Hamilton, ON, Canada; University of Toronto, Toronto, ON, Canada; Mayo Clinic, Rochester, MN.

PS-14-02  Clinico-pathological and Prognostic Difference of Screen Detected Breast Cancer Compared with Symptomatic Breast Cancer

PS-14-03  Genomic Comparison of Paired Primary Breast Carcinomas and Macrometastatic Lymph Node Metastases Using Quantitative RT-PCR by Oncotype DX® Assessment of the Recurrence Score and Quantitative Single Genes

PS-14-04  RANK Expression in Primary Tumor Tissue at the Time of Diagnosis Correlates with Risk of Subsequent Bone Metastases in the I-SPY 1 Trial (CALGB 150007/150012; ACRIN 6657)
Li J, Moore D, Yao C, Campbell M. Park J-I-SPY-1 TRIAL Investigator, Rugo HS. University of California, San Francisco, CA; Cancer and Developmental Therapeutics Program, Buck Institute for Age Research, Novato, CA.

PS-14-05  Anti-Müllerian Hormone (AMH) Levels in Premenopausal Breast Cancer Patients Treated with Adjuvant Chemotherapy - A Translational Research Project of the SUCCESS Study
Neugebauer J-K, Rack BK, Kupka M, Dinkel C, Schneeweiss A, Schrader I, Tesch H, Rezai M, Söling U, Friese K, Beckmann MW, Janin W, Müller V. Ludwig-Maximilians-Universität, Munich, Germany; University Hospital Heidelberg, Heidelberg, Germany; Henriette-Stiftung Hannover, Hannover, Germany; Facharzpraxis für Onkologie, Frankfurt, Germany; Lüsenkranzenhaus Düссeldorfer, Düссeldorfer, Germany; Gemeinschaftspraxis Sieh&Söling, Kassel, Germany; University Hospital Erlangen, Erlangen, Germany; Heinrich-Heine-Universität, Düссeldorfer, Germany; University Medical Center, Hamburg-Eppendorf, Germany.

PS-14-06  Interaction between Stoma and Tumor Characteristics as a New Prognostic and Predictive Marker in Breast Carcinomas

PS-14-07  Withdrawn

PS-14-08  Impact of Progesterone Receptor Semiquantitative Immunohistochemical Result on Oncotype DX® Recurrence Score: A Quality Assurance Study of 1078 Cases
Bhargava R, Dabbs DJ. Magee-Womens Hospital of UPMC, Pittsburgh, PA.

PS-14-09  Withdrawn

PS-14-10  Ethnic Differences in the Association between Tumor Size and Lymph Node Status among Breast Cancer Patients in South East Asia
Saxena N, Verkooyen HM, Bhoo Pathy N, Siew EL, Iau P, Lee SC, Yip CH, Hartman M. National University of Singapore, Singapore; Singapore; University Medical Center Utrecht, Utrecht, Netherlands; Julius Center University of Malaya, Kuala Lumpur, Malaysia; National Cancer Institute, National University Health Systems, Singapore, Singapore; Faculty of Medicine, University of Malaya Medical Center, Kuala Lumpur, Malaysia.

PS-14-11  Elevated Serum Ferritin Predicts Reduced Progression-Free and Overall Survival in Trastuzumab-Treated Metastatic Breast Cancer
Alkhatteeb AA, Connor J, Leitzel K, Ali S, Campbell-Baird C, Evans M, Koelster W, Fuchs E-M, Lipton A. The Pennsylvania State University Hershey Medical Center, Hershey, PA; Lebanon VA Medical Center, Lebanon, PA; Medical University of Vienna, Vienna, Austria.

PS-14-12  Bayesian Belief Network Mortality Analysis of a Breast Cancer Registry Data Set
Eberhardt JS, Hyslop T, Hu H, Rui H. DecisionQ Corporation, Washington, DC; Thomas Jefferson University, Philadelphia, PA; Windber Research Institute, Windber, PA.

PS-14-13  Favorable Prognosis in Patients with T1a,b Node-Negative Triple Negative Breast Cancers Treated with Multimodality Therapy
Ho AY, Gupta G, Perez CA, King TA, Pati SM, Rogers KH, Brogi E, Morrow M, Huds C, Traina T, McCormick B, Powell SN, Robson ME. Memorial Sloan Kettering Cancer Center, New York, NY.

PS-14-14  The Presence of a Fibrotic Focus Adds Significant Prognostic Information to the Prognostic 76-Gene Relapse Score in Lymph Node Negative Breast Cancer
Van den Eynden GG, Van Laere SJ, Smid AJ, Fooekens JA, Vermeulen PB, Dirix L.Y. Augustinus Hospital, Antwerp, Belgium; Erasmus Medical Center Rotterdam, Rotterdam, Netherlands.
PS-14-15 Prognostic Impact of Chemotherapy-Induced Amenorrhea (CIA) in Premenopausal Breast Cancer: A Meta-Analysis of Published Literatures  
Lu J, Zhou Q, Yin W. Fudan University Shanghai Cancer Center, Shanghai, China.

PS-14-16 Molecular Classification in Primary Breast Cancer and Corresponding Lymph Node Metastasis Show Impaired Prognostic Profile in the Metastatic Node  

PS-14-17 Stage IV at Presentation - Are HER2 Positive Tumors Overrepresented?  

Madsen EV, Elias SG, Gobardhan PD, van Oort PM, van der Ent FW, Nieweg OE, Valdés Olmos RA, Smeidt M, van Dalen T. Diakonessenhuis Utrecht, Utrecht, Netherlands; University Medical Centre Utrecht, Utrecht, Netherlands; The Netherlands Cancer Institute - Antoni van Leeuwenhoek Hospital, Amsterdam, Netherlands; Maastricht University Medical Centre, Maastricht, Netherlands; Orbis Medical Centre, Sittard, Netherlands.

PS-14-19 Ipsilateral Breast Tumor Recurrence Prediction with Web-Based Normogram in Korea  

PS-14-20 Neoadjuvant Chemotherapy (NCT) in 466 Patients for Operable Breast Cancer: The Prognostic Value of SBR Grade Variation  

PS-14-21 Predicting the Status of the Axillary Lymph Nodes in 1300 Consecutive Patients with Early Breast Cancer Treated in a Single Institution  

PS-14-22 Prospective Observational Study To Describe the Clinicopathological and Biological Characteristics and the Management of Metastatic Breast Cancer Patients Who Experienced Complete or Partial Remission or Disease Stabilization during at Least 3 Years  
Zamora P, Pérez-Carrón R, Manso L, Crespo C, Mendiola C, Alvarez-López I, Margeli M, Bayo-Calero JL, González-Ferre X, Santaballa A, Ciruelos EM, Afonso R, Lao J, Catalán G, Alvarez-Gallego JV, Miramón-López J, Salvador-Bofí FJ, Ruiz-Borrego M. Hospital La Paz, Madrid, Spain; Hospital Quirón, Madrid, Spain; Hospital 12 de Octubre, Madrid, Spain; Hospital Ramón y Cajal, Madrid, Spain; Hospital Donostia, San Sebastián, Spain; Hospital Germans Trias i Pujol, Badalona, Spain; Hospital Juan Ramón Jiménez, Huelva, Spain; Hospital Clinic de Barcelona, Barcelona, Spain; Hospital La Fe, Valencia, Spain; Hospital Nuestra Señora de la Candelaria, Santa Cruz de Tenerife, Spain; Hospital Miguel Servet, Zaragoza, Spain; Hospital Son Llàtzer, Mallorca, Spain; Complex Hospitalario de Zamora, Zamora, Spain; Hospital Serranía de Ronda, Ronda, Spain; Hospital Virgen de Valme, Sevilla, Spain; Hospital Virgen del Rocio, Seville, Spain.

PS-14-23 Clinical Outcomes of Different Subtypes Detected by Immunohistochemistry of Early Invasive Breast Cancers in a Monoinstitutional Series  

PS-14-24 The Long Term Prognostic Impact of Real-Time Quantitative RT-PCR Detection of Cytokeratin 19 mRNA in Preoperative Bone Marrow Aspirates of Early Breast Cancer Patients  

PS-14-25 Disease Presentation, Treatment, and Outcome in Young and Elderly Women with Breast Cancer  
Yu E, Yu D, Godette K, Mister D, Torres M. Emory University, Atlanta, GA.

PS-14-26 Results from a Prospective Clinical Study on the Impact of Oncotype DX on Adjuvant Treatment Decision Making in a Cohort of 142 UK Patients  
Holt S, Bertelli G, Brinkworth E, Durrani S, Jones S, Khawaja S, Laggner U, Moe M, Pudney D, Pitcher S, Rolles M, Sharaiah Y, Whelan S. Prince Philip Hospital, Llanelli, Carmarthenshire, United Kingdom; Singleton Hospital, Swansea, West Glamorgan, United Kingdom; Bronglais Hospital, Aberystwyth, Ceredigion, United Kingdom.

PS-14-27 Prognostic Value of Node-Negative, High Risk and 1-3 Positive Lymph Nodes Breast Cancer by Intrinsic Subtype in Patients with Adjuvant Chemotherapy  

PS-14-28 Large weight loss is associated with a higher risk of mortality in breast cancer survivors with comorbid conditions  
Cain B, Shu X-O, Chen W, Pierce J, Kwan M, Kroenke C, Patterson R, Nechuta S. Division of Research, Kaiser Permanente, Oakland, CA; Vanderbilt University, Nashville, TN; Harvard Medical School, Boston, MA; University of California, San Diego, San Diego, CA.

PS-15-01 Words Matter: Influence of DCIS Diagnosis Terminology on Patient Treatment Decisions  
Omer Z, Hwang ES, Esserman LJ, Ozanne EM. University of California, San Francisco; Massachusetts General Hospital.

PS-15-02 Patients’ Views about How Oncologists Should Explain Prognosis in Advanced Cancer  
Kielty BE, McCaughan G, Christodoulou S, Tattersall MHN, Beale P, Grimison P, Stockler MR. University of Sydney, Sydney, NSW, Australia; Royal Prince Alfred and Concord Hospitals, Sydney, NSW, Australia.

PS-15-03 Development of a Patient Decision Aid for Women 70 Years and Older with Stage I, Hormonally Sensitive, Breast Cancer Considering Adjuvant Treatment Post-Lumpectomy  
Szumacher E, Wong J, D’Allimonte L, Angus J, Paszat L, Metcalfe K, Whelan T, Llewellyn-Thomas H. Odette Cancer Centre, Sunnybrook Health Sciences Centre, Toronto, ON; Canada; University of Toronto, Toronto, ON; CAN; Canada; Education Independent Consultant, Toronto, ON; Juravinski Cancer Centre, Hamilton, ON; Canada; Women’s College Research Institute, Toronto, ON; Canada; Dartmouth Medical School, Lebanon, NH.

PS-15-04 Withdrawn
Impact of Breast Specialized Physician Consultation on Surgical Decision Making for Breast Cancer and Decision Conflict in Women with Hereditary Breast Cancer Risk
Boughey JC, Hoskin TL, Williams CJ, Hartmann LC, Allers TM, Degnim AC, Frost M. Mayo Clinic, Rochester, MN.

PS-15-02 The Impact of Educational Materials on Compliance and Persistence with Adjuvant Aromatase Inhibitors: 2 Year Follow-Up and Final Results from the CARAT/IDE Study
Neven P, Markopouloos C, Tanner MME, Marty ME, Kreienberg R, Atkins L, Franquet AA, Serin D, Gulcelik MA, Deschamps V. University Hospitals Leuven, Leuven, Belgium; Medical School University of Athens, Athens, Greece; Tampere University Hospital, Tampere, Finland; Saint Louis University Hospital, Paris, France; University of Ulm, Ulm, Bade-Wurttemberg, Germany; University College London, London, United Kingdom; IDDI (International Drug Development Institute), Louvain-la-Neuve (Ottignies), Belgium; Institut St. Catherine, Avignon, France; Ankara Oncology Hospital, Ankara, Turkey; Astra Zeneca Germany, Wedel, Germany; Aledis GmbH, Germany; Hospital Rosenheim, Rosenheim, Germany; Hospital St. Georg, Leipzig, Germany; Consulting Doctor-Patient Communication, Radolfzell, Germany; University Women's Hospital, Ulm, Germany.

PS-15-03 The Breast Cancer Novela, Se Valiente…Son Tus Senos, an Innovative Tool To Educate Latina Women about Breast Cancer

Evaluation of Psychosocial Distress in Main Care-Givers of Patients with Metastatic Breast Cancer Who Receive Treatment in a Community Based Oncology Group Practice
Weide R, Feiten S, Friessenhahn V, Heymanns J, Klebath K, Mengenthaler U, Thomalla J, van Roye C, Köppler H. Hematology/Oncology Group Practice, Koblenz, Germany; Institute for Health Research in Oncology, Koblenz, Germany

Associations between Breast Cancer Patients' Satisfaction with Nursing Staff and Hospital Characteristics, Results of a German Multicenter Study
Wuerstlein R, Kowalski C, Diener S, Krebs S, Pfaff H, Harbeck N. University Hospital Cologne, CIO, Köln, Germany.

The Association between Breast Cancer Related Lymphedema's Risk Factors and Likelihood of Edema Progression
Skolny MN, Miller CL, O'Toole J, Sadek B, Ancukiewicz M, Taghian AG. Massachusetts General Hospital, Boston, MA.

Association between Breast Cancer and Osteoporosis among Women 85 Years or Older
Okanami Y, Honma N, Arai T, Sawabe M, Maeda I, Takagi M, Younes M, Takubo K. St. Marianna University School of Medicine, Kawasaki, Kanagawa, Japan; Tokyo Metropolitan Institute of Gerontology; Baylor College of Medicine.

PS-17-05 24 Months Follow-Up Results from PACT (Patient's Anastrozole Compliance to Therapy Programme), a Non-Interventional Study Evaluating the Influence of a Standardized Information Service on Compliance in Postmenopausal Women with Early Breast Cancer
Lueck H-J, Hadsj P, Harbeck N, Jackisch C, Blettner M, Zau N, Windemuth-Kieselbach C, Beck T, Köhler U, Schmitt D, Kreienberg R. Gyn-Oncological Practice, Hannover, Germany; Phillips-University, Marburg, Germany; Breast Center, University of Cologne, Cologne, Germany; City Womens Hospital Offenbach, Offenbach, Germany; Institute of Medical Biostatistics, University, Mainz, Germany; AstraZeneca Germany, Wedel, Germany; Aledis GmbH, Germany; Hospital Rosenheim, Rosenheim, Germany; Hospital St. Georg, Leipzig, Germany; Consulting Doctor-Patient Communication, Radolfzell, Germany; University Women's Hospital, Ulm, Germany.

PS-17-06 Pilot Study of a Questionnaire To Assess Impact of a Breast Cancer Diagnosis in Young Women on Their Relationship with Their Mothers
Ab A, Ferguson K, Wright F, Pritchard K, Kiss A, Warner E. Sunnybrook Health Sciences Centre, Toronto, ON, Canada.

Her2+ Metastatic Breast Cancer Patient Experiences on Treatment in the Biologic Era: Findings from a Community Web-Based Survey
Mayer M, Doan JF, Lang K, Hurvitz SA, Lalla D, Woodward RM, Brammer MG, Menzin J, Tripathy D. AdvancedBC.org; Genentech, Inc., South San Francisco, CA; Boston Health Economics, Waltham, MA; UCLA Jonsson Comprehensive Cancer Center, Los Angeles, CA; USC/Norris Comprehensive Cancer Center, Los Angeles, CA.

The Value of Progression-Free Survival from the Patient Perspective: An Online Survey of Women with Metastatic Breast Cancer in the United States
Hurvitz SA, Mathias SD, Doan JF, Crosby RD. UCLA School of Medicine/Translational Oncology Research International, Los Angeles, CA; Health Outcomes Solutions, Winter Park, FL; Genentech, Inc., South San Francisco, CA.

Treatment – Chemotherapy: Adjuvant Chemotherapy
Truini W, Voogd A, Vreugdenhil G, Van der Heiden-van der Loo M, Sieuling S, Roumen R. Maxima Medical Centre, Veldhoven, Netherlands; Maastricht University, Maastricht, Netherlands; Comprehensive Cancer Centre, Utrecht, Netherlands; Comprehensive Cancer Centre, Enschede, Netherlands.

PS-18-01 No Effect of Adjuvant Chemotherapy in Postmenopausal Patients with Invasive Lobular (Mixed) Breast Cancer
Truini W, Voogd A, Vreugdenhil G, Van der Heiden-van der Loo M, Sieuling S, Roumen R. Maxima Medical Centre, Veldhoven, Netherlands; Maastricht University, Maastricht, Netherlands; Comprehensive Cancer Centre, Utrecht, Netherlands; Comprehensive Cancer Centre, Enschede, Netherlands.

A Population Level Assessment of Emergency Room Visits and Hospitalizations for Women Undergoing Adjuvant Chemotherapy for Early Breast Cancer
Enright KA, Trudeau M, Yun L, Grunfeld E, Krzyzanowska M. Peel Regional Cancer Centre, Credit Valley Hospital, Mississauga, ON, Canada; Sunnybrook Odette Cancer Centre, Toronto, ON, Canada; Institute for Clinical Evaluative Science, Toronto, ON, Canada; University of Toronto, Toronto, ON, Canada; Cancer Care Ontario, Toronto, ON, Canada; Princess Margaret Hospital, Toronto, ON, Canada.
December 6-10, 2011  
Program Schedule

PS-18-03  First Interim Toxicity Analysis of the Randomized Phase III WSG Plan B Trial Comparing 4xE C-4xD doc Versus 6xTC in Breast Cancer Patients with HER2 Negative Breast Cancer (BC)
Nitz U, Gluz O, Krepe H, Liedtke B, Aktas B, Herschens S, Pollmanns A, Krabisch P, Zuna L, Shvak S, Thomssen C, Harbeck N, West German Study Group, Moenchengladbach, Germany; Breast Centre Niederer Ben/Bethesda Hospital, Moenchengladbach, Germany; Bethesda Hospital, Wuppertal, Germany; Medizinische Hochschule, Hannover, Germany; Evangelical Hospital, Bergisch Gladbach, Germany; University Hospital, Essen, Germany; Johanniter Hospital, Stendal, Germany; Evangelical Hospital, Oberhausen, Germany; City Hospital Chemnitz, Chemnitz, Germany; Genomic Health Inc, Redwood City, CA; University Hospital Halle/Saale, Halle/Saale, Germany; University Hospital Cologne, Cologne, Germany.

PS-18-04  Safety Profile of Ixabepilone as Adjuvant Treatment for Poor Prognosis Early Breast Cancer: First Results of theunicancer-PACS 08 Trial
Cammone M, Spielmann M, Wildiers H, Cottu P, Kerbrat P, Levy C, Mayer F, Bachelot T, Winston T, Eymard J-C, Uwer L, Machels J-P, Verhoeven D, Jaubert F, Facchin T, Orfeuvre H, Canon J-L, Asselin B, Roca L, Lacroix Tiki M, Martin AL, Roche H, Centre René Gauducheur, Nantes, France; Institut Gustave Roussy, Villejuif, France; Catholique Universit, Leuven, Belgium; Institut Curie, Paris, France; Centre Éugène Marquis, Rennes, France; Centre François Baclesse, Caen, France; Centre Georges-François Leclerc, Dijon, France; Centre Léon Bérard, Lyon, France; Mayo Clinic Florida, Jacksonville; Institut Jean Godinot, Reims, France; Centre Alexis Vautrin, Nancy, France; UCL Cliniques Universitaires Saint-Luc, Bruxelles, Belgium; AZ Klin A Oncology, Brasschaat, Belgium; Clinique Tivoli, Bordeaux, France; Polyclinique de Courlancy, Reims, France; Centre Hospitalier de Fleyriat, Bourg en Bresse, France; Grand Hopital de Charleroi, Charleroi, Belgium; Centre Val d’Aurelle, Montpellier, France; Institut Claudius Regaud, Toulouse, France; R&D Unicancer, Paris, France.

PS-18-05  Incidence of Febrile Neutropenia in Patients Treated with Docetaxel and Cyclophosphamide (TC) for Adjuvant Breast Cancer

PS-18-06  Taxanes and Cyclophosphamide Are Equally Effective in Breast Cancer: A Meta-Analysis of Ten Phase III Trials in Early and Advanced Disease
Vriens BE, Lobbezoo DJ, Voogd AC, Veenk J, Tjan-Heijnen VC. Maastricht University Medical Centre, Maastricht, Netherlands; Maxima Medical Centre, Eindhoven, Netherlands.

PS-18-07  Presence of Disseminated Tumor Cells after Adjuvant Chemotherapy in Breast Cancer and Disseminated Tumor Cells Monitoring during Secondary Adjuvant Treatment
Synnestvedt M, Borgen E, Wist E, Wiedswang G, Weyde K, Risberg T, Kersten C, Nygaard I, Vindi L, Schirmer CB, Nesland JM, Naume B. Oslo University Hospital, The Radium Hospital, Oslo, Norway; Oslo University Hospital, Ullevål, Oslo, Norway; Hospital Imlandet, Gjøvik, Norway; University Hospital Northern Norway, Tromsø, Norway; Sørlandet Hospital Kristiansand, Kristiansand, Norway; Stavanger University Hospital, Stavanger, Norway; Ålesund Hospital, Ålesund, Norway; Stavanger University Hospital, Stavanger, Norway; Roche, France; Institut Gustave Roussy, Villejuif, France.

PS-18-08  A Comparative Effectiveness Analysis of Trastuzumab Persistence between Two Adjuvant Breast Cancer Treatment Regimens among US Health Plan Enrollees

PS-18-09  The Incidence of Febrile Neutropenia in the First Course of Adjuvant Chemotherapy with Docetaxel/Cyclophosphamide with or without Pegfilgrastim
Jones S, Paul D, Sledacter S, Vukelja S, Wilks LT, Stokoe C, Osborne CR, Kerekow L, McIntyre K, Holmes FA, Guerra LG, Zhan F, Asmar L, O’Shaugnessy J, Blum JL. US Oncology, The Woodlands, TX; Baylor-Sammons Cancer Center, Baylor University, Dallas, TX; Rocky Mountain Cancer Center, Denver, CO; Texas Oncology-Tyler, Tyler, TX; Cancer Care Center of South Texas, San Antonio, TX; Texas Oncology, Plano, TX; Breast Cancer Center of North Texas, Bedford, TX; Texas Oncology-Dallas Presbyterian Hospital, Dallas, TX; Texas Oncology-Houston Memorial City, Houston, TX.

PS-18-10  Utilisation of Primary and Secondary G-CSF Prophylaxis Enables Optimal Dose Delivery of Standard Adjuvant Chemotherapy in Early Breast Cancer: Results of 1653 Patients from a Single Institution
Chan A, McGreggor S. Mount Hospital, Perth, WA, Australia.

PS-18-11  Incidence of Chemotherapy Dose Reductions and Dose Delays, and Reduced Chemotherapy Dose Intensity in Early Stage Breast Cancer
Weycker D, Edelberg J, Kartashov A, Barron R, Lyman G. Policy Analysis Inc. (PAI), Brookline, MA; Amgen Inc., Thousand Oaks, CA; Duke University, Durham, NC.

PS-18-12  Perception, Practice and Toxicity of Adjuvant Treatment of HER2+ Breast Cancer in Wisconsin
Rocque GB, Onitilo AA, Engel JM, Pettite EN, Boshoven AM, Zhang S, Kim K, Rishi S, Waack B, Winsinski KB, Tevaarwerk AJ, Burkard ME. University of Wisconsin Carbone Cancer Center, Madison, WI. The Marshfield Clinic, Weston, WI; University of Wisconsin School of Medicine and Public Health, Madison, WI.

PS-18-13  Adjuvant Chemotherapy with Vinorelbine+5FU or Capecitabine in Poor Responders to Neoadjuvant EC-Docetaxel Chemotherapy (NAC) for Locally Advanced Breast Cancers

Treatment – Chemotherapy: Chemotherapy in Advanced Disease

Campone M, Dobrovoloskaya N, Tjulandin S, Chen S-C, Fourie S, Mefti M, Konstantinova M, Lefresne F, Meheust N, Jassem J. Institut de Cancérologie de l’Ouest/René Gauducheur, Saint Herblain, Nantes Cedex, France; Cancer Research Center of Roentgenomarology, Moscow, Russian Federation; Russian Oncological Research Center, Moscow, Russian Federation; Chang-Gung Memorial Hospital, Taipei, Taiwan; Wilmed Park Oncology, Klerkdoor, South Africa; Centre René Huguenin, Saint-Cloud, France; Moscow Regional Oncology Dispensary, Moscow, Russian Federation; University Hospital, Essen, Germany; Johanniter Hospital, Hamburg, Germany; Evangelical Hospital, Bergisch Gladbach, Germany; Betheslada University, Wuppertal, Germany; Medizinische Hochschule, Hannover, Germany; Evangelical Hospital, Bergisch Gladbach, Germany; University Hospital, Essen, Germany; Johanniter Hospital, Stendal, Germany; Evangelical Hospital, Oberhausen, Germany; City Hospital Chemnitz, Chemnitz, Germany; Genomic Health Inc, Redwood City, CA; University Hospital Halle/Saale, Halle/Saale, Germany; University Hospital Cologne, Cologne, Germany.

www.aacrjournals.org
Comparison of the Incidence of Peripheral Neuropathy with Eribulin Mesylate Versus Ixabepilone in Metastatic Breast Cancer Patients: A Randomized Phase II Study

Vahdat L, Gopalakrishna P, Garcia AA, Vogel C, Pellegrino C, Lindquist, Jannotti N. Weill Cornell Medical College, New York, NY; Eisai, Inc., Woodcliff Lake, NJ; University of Southern California Keck School of Medicine, Los Angeles, CA; Sylvestre Comprehensive Cancer Center, University of Miami, Miller School of Medicine, Deerfield Beach, FL; Montefiore Medical Center, New York, NY; Hematology-OncoLOGY-Associates of the Treasure Coast, Port Saint Lucie, FL; US Oncology, Sedona, AZ.

Albumin-Bound Paclitaxel (ab-pac) Versus Docetaxel for First-Line Treatment of Metastatic Breast Cancer (MBC): Overall Survival and Safety Analysis of a Randomized Phase II Trial

Gradishar WJ, Krasnojon D, Cheporov S, Makhson AN, Manikhas GM, Clawson A, Bhat P. Northwestern University, Chicago, IL; Leningrad Regional Oncology Center, Russian Federation; Yaroslav Regional Clinical Oncology Hospital, Yaroslavl, Russian Federation, City Oncology Hospital, Moscow, Russian Federation; St. Petersburg Oncology Center, St. Petersburg, Russian Federation; Celgene Corporation, Summit, NJ.

Results of a Randomized Phase II Study Demonstrate Benefit of Platinum-Based Regimen in the First-Line Treatment of Triple Negative Breast Cancer (TNBC)

Fan Y, Xu BH, Yuan P, Wang JY, Ma F, Ding XY, Zhang P, Li Q, Cai RG. Cancer Institute & Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, China.

Age-Related Changes in the Pharmacokinetics (pk), Response, and Toxicity of Weekly nab-Paclitaxel in Patients with Metastatic Breast Cancer (MBC)


Highly Effective of Gemcitabine and Cisplatin (gp) as First-Line Combination therapy in patients with Triple-Negative Breast Cancer: Final Report of a Phase II Trial


The Effects of ABC2 and SLCO1B3 Single Nucleotide Polymorphisms on Docetaxel-Induced Leukopenia in Breast Cancer Patients

Chen J, Wu LJ, Shen P, Yu LF, Huang S. The First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, Zhejiang, China.

Comparison of Individual Patient Data from Capecitabine Monotherapy Clinical Trials in Anthracycline-/Taxane-Pretreated Locally Advanced or Metastatic Breast Cancer (LA/MBC)

Blum JL, Barrios CH, Feldman N, Verma S, McKenna E, Lee S, Scotto N, Gralow J. Baylor Sammons Cancer Center, Texas Oncology, US Oncology, Dallas, TX; PUCRS School of Medicine, Porto Alegre, Brazil; Olive View-UCLA Medical Center, Sylmar, CA; Sunnybrook Odette Cancer Centre, University of Toronto, Toronto, ON, Canada; Hoffmann-La Roche Inc., San Francisco, CA; Hoffmann-La Roche Ltd, Basel, Switzerland; University of Washington, Seattle, WA.

Phase II Trial of Ixabepilone Plus Carboplatin in Patients with Metastatic Breast Cancer: The ECLIPSE Study

Osborne C, Challagulla JD, Fanning SR, Eisenhors CF, Holmes FA, Monaghan G, Neubaurer MA, Rabe AC, Raja V, Robbins GJ, Taboada C, Vukelja SJ, Wilks ST, Wang Y, Brown J-A, Asmar L, O'Shaughnessy J. US Oncology, The Woodlands, TX; Baylor-Sammons Cancer Center, Dallas, TX; Texas Oncology, Witchita Falls, KS; Cancer Centers of The Carolinas, Greenville, SC; Cancer Centers of North Carolina, Raleigh, NC; Texas Oncology-Houston Memorial City, Houston, TX; Kansas City Cancer Center, Kansas City, MO; Kansas City Cancer Center-Southwest, Overland Park, KS; Kansas City Cancer Center, Kansas City, KS; Florida Cancer Institute-New Hope, New Port Richey, FL; Texas Oncology-Methodist Charlton Cancer Center, Dallas, TX; Texas Oncology-Tyler, Tyler, TX; Cancer Centers of South Texas, San Antonio, TX.

Correlation of Response of Weekly Paclitaxel and Paraplatin as First Line Treatment of Metastatic Breast Cancer with p53 Status

El-Sadda W, Magdy M, Abdel-Halim I, Abdel-Wahab A. Mansoura University Hospital, Mansoura, Egypt.

Tesetaxel, an Oral Taxane, as First-Line Therapy for Women with Metastatic Breast Cancer

Schwartzberg L, Rubin P, Patnaik A, Itri L, Olson AL, Seidman AD. The West Clinic, Memphis, TN; The Moses H. Cone Regional Cancer Center, Greensboro, NC; START Center - South Texas Accelerated Research Therapeutics, San Antonio, TX; Genta Incorporated, Berkeley Heights, NJ; Memorial Sloan-Kettering Cancer Center, New York, NY.

Integration of Capecitabine Monotherapy with Capecitabine Combination Therapy in Metastatic Breast Cancer Patients: First Report on Safety and Efficacy of Single Agent Capecitabine Maintenance Study


A Randomized Phase II Trial of First-Line Metastatic Breast Cancer (MBC) Patients: Sub-Set Analysis of Albumin-Bound Paclitaxel (ab-pac) Given Weekly at 150 mg/m2

Gradishar WJ, Krasnojon D, Cheporov S, Makhson AN, Manikhas GM, Clawson A, Bhat P. Northwestern University Feinberg School of Medicine, Chicago, IL; Leningrad Regional Oncology Center, Russian Federation; Yaroslav Regional Clinical Oncology Hospital, Yaroslavl, Russian Federation; City Oncology Hospital, Moscow, Russia; City Oncology Hospital, St. Petersburg, Russian Federation; Yaroslavl Regional Clinical Oncology Hospital, Yaroslavl, Russian Federation; City Oncology Hospital, Russian Federation; St. Petersburg Oncology Center, St. Petersburg, Russian Federation; Celgene Corporation, Summit, NJ.

Platinum-Based Chemotherapy in Triple-Negative Breast Cancer

Villarreal-Garza C, Bouganim N, Khalaf D, Clemons M, Kassam F, Enright K, Verma S, Myers J, Dent R. Sunnybrook Odette Cancer Centre, Toronto, ON, Canada; The Ottawa Hospital Cancer Centre, Ottawa, ON, Canada; Princess Margaret Hospital, Toronto, ON, Canada; Credit Valley Hospital, Mississauga, ON, Canada.

Treatment – Chemotherapy: Toxicities and Management

Patient Valuation of Reduced Risk of Side Effects during Treatment for Metastatic Breast Cancer

Lalla D, McLaughlin T, Brummer M, Bramley T, Bare A, Carlton R, Genentech Inc., S San Francisco, CA; Xcenda, Palm Harbor, FL.

N-Terminal Pro-Brain B-Type Natriuretic Peptide (nt-pro-BNP) before and during Treatment with Trastuzumab Allows Early Detection of Cardiotoxicity in Breast Cancer Patients

Blancas I, Camillo J, Legérin M, Delgado M, Jurado JM, Zarcos I, Villaescusa A, Gómez FJ, Moreno E, García-Puche JL. Hospital Clínico San Cecilio, Granada, Spain; University of Granada, Granada, Spain.
Personalizing Supportive Care: A Clinical Prediction Model for Neutropenic Complications in Patients with Early-Stage Breast Cancer (ESBC) Receiving Intermediate Risk Chemotherapy
Kudeerer NM, Culakova E, Poniewierski MS, Crawford J, Dale D, Lyman GH. Duke University, Durham, NC; University of Washington, Seattle, WA.

Is Primary Prophylaxis with G-CSF Indicated for Adjuvant TC or FEC-D Chemotherapy? A Systematic Review and Meta-Analyses
Younis T, Rayson D, Thompson K. Dalhousie University at the Queen Elizabeth II Health Sciences Centre, Halifax, NS, Canada.

Peripheral Blood Transcriptomics and Doxorubicin Cardiotoxicity
Todorova VK, Beggs ML, Dhakal IB, Hennings LJ, Mahkoul I, Klimger VS. University of Arkansas for Medical Sciences, Little Rock, AR.

Survival in Women with Breast Cancer Who Used or Did Not Use Scalp Cooling in the Neoadjuvant/Adjuvant Setting
Lemieux J, Perron L, Provencher L, Brisson J, Amireault C, Blanchette C, Maussell E. Centre de Recherche FRQS du CHA Universitaire de Québec, Quebec City, QC, Canada; Hôpital du Saint-Sacrement, Quebec City, QC, Canada; Université Laval, Quebec City, QC, Canada; Institut National de Santé Publique, Quebec City, QC, Canada; Université de Montréal, Montreal, QC, Canada.

Estimation of Febrile Neutropenia in Women Receiving Docetaxel Plus Cyclophosphamide as Adjuvant Therapy for Early Stage Breast Cancer: A Retrospective Analysis

Multicenter Results of Scalp Cooling To Prevent Chemotherapy-Induced Alopecia in 1500 Breast Cancer Patients
van den Hurk C, Peeboom M, Komen M, Nortier H, Breed W. Comprehensive Cancer Centre South (IKZ), Eindhoven, Netherlands; Medical Centre Alkmaar, Alkmaar, Netherlands; Leiden University Medical Centre, Leiden, Netherlands.

Treatment – Trial Resources etc.: Tissue and Data Banks

A Renewable Tissue Resource of Phenotypically Stable Human Breast Cancer Xenografts for Preclinical Studies
Zhang X, Dobrolecki LE, Lai Q, Landis MD, Wong H, Tsimelzon A, Claerhout S, Contreras A, Gutierrez C, Huang J, Wu M-F, Pavlick AC, Froehlich AM, Hilsenbeck SG, Mills GB, Wiechmann L, Petrovic I, Rimawi MF, Schiff R, Chang JC, Lewis MT. Baylor College of Medicine, Houston, TX; The University of Texas MD Anderson Cancer Center, Houston, TX.

The Susan G. Komen for the Cure® Tissue Bank at the IU Simon Cancer Center: The Source for Normal Breast Tissue and Biospecimens
Clare SE, Mathieson T, Henry JE, Zhang H, Way ES, Ridley KE, Badve S, Herbert B-S, Rufenbarger CA, Storniolo AMV. Indiana University School of Medicine, Indianapolis, IN.

The Breast Cancer Campaign Tissue Bank
Jones L, Chelala C, Ellis E, Ekbote J, Green A, Hanby A, Jordan L, Purdie C, Quinlan P, Speirs V. Barts Cancer Institute, London, United Kingdom; University of Nottingham, Nottingham, United Kingdom; University of Leeds, Leeds, United Kingdom; University of Dundee, Dundee, United Kingdom.

Patient Attitudes towards Undergoing Additional Breast Biopsy for Research Purposes
Nairn FM, Ballinger R, McLaughlan R, Hadjiminas DJ, Hogben K, Palmieri C, Cleetor SJ. Royal Free Hospital, Royal Free Hampstead NHS Trust, London, United Kingdom; Brighton and Sussex Medical School, Brighton, United Kingdom; Charing Cross Hospital, Imperial NHS Healthcare Trust, London, United Kingdom.

Feasibility and Patient Safety of Serial Biopsies (bx) in Metastatic HER2-Positive Breast Cancer (BC) To Evaluate Alterations in Molecular Biomarkers (BM): Preliminary Results of SHERSig (Study of HER2 Signature in Metastatic Breast Cancer) a Prospective Phase II Study
Chan A, Chan S, Price D, Bergh J, Uch J, Redfern A, Chirgwin J, Librindo E, Dhadda A, Lopez-Vega J, Lindman B, Bith L, Barn-Hay S, Kiemier A, Herbst F, Ellis I. Mount Medical Centre, Perth, Australia; Nottingham City Hospital, Nottingham, United Kingdom; Karolinska Institutet and University Hospital, Stockholm, Sweden; Hospital Clinico Universitario de Valencia, Valencia, Spain; Royal Perth Hospital, Perth, Australia; Eastern Health Melbourne, Australia; Scarborough Hospital, Scarborough, United Kingdom; Hospital Universitario Marques de Valdecilla, Santander, Spain; Uppsala University Hospital, Uppsala, Sweden; Royal Prince Alfred Hospital, Camperdown, Australia; Royal North Shore Hospital, St Leonards, Australia; F Hoffmann-La Roche Ltd, Basel, Switzerland; Nottingham University Hospitals, Nottingham, United Kingdom.

The Impact of Primary Tumor Resection on the Survival of Patients with Stage IV Breast Cancer According to Molecular Subtype
Ahn SK, Moon H-G, Kim JS, You Jm, Shin Hc, Han W, Noh D-y. Seoul National University Hospital.

Clinicopathological Features of Young Patients Age <35 Years with Breast Cancer in Japan
Katoaka A, Tokunaga E, Masuda N, Shien T, Ohno S, Kinoshita T, Shimizu C. Breast Surgery Clinic, Minato-ku, Tokyo, Japan; Kyushu University Hospital, Fukuoka, Japan; National Hospital Organization Osaka National Hospital, Osaka City, Osaka, Japan; Okayama University Hospital, Okayama City, Okayama, Japan; National Hospital Organization Kyushu Cancer Center, Fukuoka City, Fukuoka, Japan; National Cancer Center Hospital, Chuo-ku, Tokyo, Japan; The Japanese Breast Cancer Society (JBCS) Study Group on Breast Cancer in Young Women.

Breast Cancer in Elderly Treatment Algorithm - A New Approach To Optimize the Management of Breast Cancer in Older Patients
Tahir M, Pretorius R, Robinson T, Walker R, Stottter A. University Hospitals of Leicester NHS Trust, Leicester, United Kingdom.

Gonadotrophin-Releasing Hormone Analogues for Ovarian Function Preservation in Women with Premenopausal Breast Cancer Undergoing Adjuvant Chemotherapy: A Systematic Review and Meta-Analysis
Cruz MRS, Motta E, Silva EMK, Atallah AHN. Hospital Sao Jose, Sao Paulo, Brazil; Universidade Federal de Sao Paulo, Sao Paulo, Brazil.

Withdrawn

Monitoring of Quality Indicators Should lead to Quality Improvement: A Practical Guide for Clinical Trials

Treatment Strategy of Locally Advanced Breast Cancer in Sub Group (T3N1M0, T4bN1M0) in Developing Countries
Gupta AK, Kaushal M. Mahatma Gandhi Memorial Medical College and Maharaja Yashwantrao Hospital, Indore, Madhya Pradesh, India.
PS-23-08  Minimally invasive treatment of elderly breast cancer patients using preferential radiofrequency ablation
Schasbiger K-LJ, Lofgren L, Leifland K, Thomsen K, Wiksell H. Karolinska Institutet, Stockholm, Sweden; Capio St Gorans Hospital, Stockholm, Sweden; Unilabs AB, Capio St Gorans Hospital, Stockholm, Sweden.

5:00 pm–7:00 pm
POSTER DISCUSSION IX: PI3K/MTOR
Ballroom A
Viewing 5:00 pm
Discussion 5:30 pm
Discussant: Ana-Maria Gonzalez-Angulo, MD, MSC
UT MD Anderson Cancer Center
Houston, TX

PD09-01 Target-Based Therapeutic Matching in Early-Phase Clinical Trials in Patients with Advanced Breast Cancer and PI3CA Mutations
Janku F, Moulder SL, Wheler JJ, Stepanek V, Falchuck GS, Naing A, Hong DS, Fu S, Piha-Paul SA, Luthra R, Tsmbriendou AM, Kurzrock R. The University of Texas MD Anderson Cancer Center, Houston, TX.

PD09-02 Withdrawn

PD09-03 Phase I/II Study of BMK120 in Combination with Trastuzumab in Patients with HER2 Overexpressing Metastatic Breast Cancer Resistant to Trastuzumab-Containing Therapy
Saura C, Bendell J, Jerusalem G, Graña-Suárez B, Su S, Su Q, De Buck S, Devisse C, Bosch A, Urtucuchoea A, Beck JT, DiTomaso E, Rouyze N, Stemberg DW, Massacesi C, Hirawat S, Dinx L, Baselga J. Hospital Vall d’Hebron, Barcelona, Spain; Sarah Cannon Research Institute, Nashville, TN; C.H.U. Sant-Tilman, Liege, Belgium; Novartis Pharmaceuticals, Horsham Park, NJ; Novartis Pharma AG, Basel, Switzerland; Hospital Clinico Universitario de Valencia, Valencia, Spain; Catalan Institute of Oncology, Barcelona, Spain; Highlands Oncology Group, Fayetteville, AZ; Novartis Institutes for BioMedical Research, Cambridge, MA; Novartis Oncology, Paris, France; Oncologisch Centrum AZ-St. Augustinus Oncology, Wilrijk, Belgium; Massachusetts General Hospital, Boston, MA.

PD09-04 A Phase Ib, Open-Label, Dose-Escalation Study of the Safety and Pharmacology of the PI3-Kinase Inhibitor GDC-0941 in Combination with Paclitaxel and Bexacizumab in Patients with Locally Recurrent or Metastatic Breast Cancer
Scholfski P, De Benedictis E, Gendreau S, Gennai L, Krop IE, Levy G, Ware J, Wildiers H, Winer EP. Catholic University, Leuven, Belgium; Istituto Nazionale dei Tumori, Milan, Italy; Gencentech Inc., South San Francisco, CA; Dana-Farber Cancer Institute, Boston, MA.

PD09-05 SU2C Phase Ib Study of pan-PI3K Inhibitor BMK120 Plus Aromatase Inhibitor Letrozole in ER+/HER2- Metastatic Breast Cancer (MBC)
Mayer IA, Ballo JM, Kuba MG, Sanders ME, Yap J, Li Y, Winer E, Arteaga CL. Vanderbilt-Ingram Cancer Center, Nashville, TN; Dana Farber Cancer Institute, Boston, MA; MD Anderson Cancer Center, Houston, TX.

PD09-06 Phase II Trial of RAD001 (Everolimus), an mTOR Inhibitor, with Weekly Cisplax and Paclitaxel in Patients with HER2-Negative Metastatic Breast Cancer (MBC)
Mayer IA, Means-Powell J, Abramson VG, Shyr Y, Baliok JM, Kuba MG, Ghavri HM, Schlabach L, Arteaga CL, Pietenpol JA. Vanderbilt-Ingram Cancer Center, Nashville, TN; Vanderbilt-Ingram Cancer Center Affiliated Network (VICIAN).

PD09-08 Combined Inhibition of mTORC1 with Temsirolimus and HER2 with Neratinib: A Phase II/I Study in Patients with Metastatic HER2-Amplified or Triple-Negative Breast Cancer

5:00 pm–7:00 pm
POSTER DISCUSSION X: TUMOR SUPPRESSORS/DNA REPAIR
Ballroom B
Viewing 5:00 pm
Discussion 5:30 pm
Discussant: Nicholas Turner, MD
Royal Marsden Hospital
London, UNITED KINGDOM

PD10-01 Prevalence of Dysfunctional Fanconi Anemia (FA) DNA Repair Pathway in Breast Cancer
Ramswamy B, Strividya V, Mullins D, Carothers S, Young G, Wernui D, Zhao W, Lustberg M, Leon M, Wleslowski R, Layman R, Mrozek E, Shapiro CV, Villalona-Calero M. The Ohio State University, Columbus, OH.

PD10-02 Sporadic Breast Cancers Show Defects in the BRCA1-BRCA2 Pathway of Homologous Recombination in All Biomarker-Defined Sub-Types of Breast Cancer
Powell SN, Mutter RW, Deliste R, Bindra R, King T, Giri D, Park J. Memorial Sloan-Kettering Cancer Center, New York, NY.

PD10-03 Withdrawn

PD10-04 Exploration of the Relationship between Loss of PTEN and BRCA1 Expression in Triple Negative Breast Carcinoma

PD10-05 Identification of Novel BRCA1 Transcriptional Targets That Promote the Survival of BRCA1-Mutated Estrogen Receptor-a Negative (ER-a) Breast Tumours
Lamers E, Haddock P, Cochrane DJ, Gorski JJ, Blayney J, McDeray FA, Mullan JM, Mullan PB, Couch FJ, Kennedy RD, Harkin PD, Quinn JE. Queen’s University of Belfast, Belfast, Antrim, United Kingdom; Almac Ltd., Craigavon, United Kingdom; Mayo Clinic College of Medicine, Rochester, MN.

PD10-06 Loss of the Retinoblastoma Tumor Suppressor (RB) in Triple Negative Breast Cancer Is Associated with a Favorable Prognosis

PD10-07 microRNA Profiles of Breast Tumors Identifies the mir 17-92 Cluster as a Group of Potentially Essential Oncomirs in Triple-Negative Breast Cancer
Son BH, Birkbak NJ, Tian R, Iglehart D, Wang ZC, Richardson AL. Dana-Farber Cancer Institute, Boston, MA; Asian Medical Center, Seoul, Korea; Technical University of Denmark, Lyngby, Denmark; Brigham and Women’s Hospital, Harvard Medical School, Boston, MA.

5:00 pm–7:00 pm
POSTER SESSION ONGOING TRIALS 3
Exhibit Hall B

New Agents
OT3-01-01 Randomized Phase II Study of Fulvestrant Versus Fulvestrant Plus Bortezomib in Postmenopausal Women with Estrogen Receptor (ER) Positive, Aromatase-Inhibitor (AI) Resistant Metastatic Breast Cancer (MBC): New York Cancer Consortium Trial P8457
Adelson KD, Raptis G, Sparano J, Germain D. Mount Sinai School of Medicine, New York, NY; Albert Einstein Cancer Center, Bronx, NY.
OT3-01-02
Imatinib Mesylate in Combination with Vinorelbine for Patients with Metastatic Breast Cancer - An Ongoing Phase I/II Clinical Trial

OT3-01-03
Pre-Surgical Evaluation of the AKT Inhibitor MK-2206 in Patients with Operable Invasive Breast Cancer: New York Cancer Consortium Trial PI840
Kalinsky K, Sparano JA, Kim M, Crew KD, Maurer MA, Tabak B, Feldman SM, Hishiboshi H, Wiechmann LS, Adelson KB, Hershman DL. Columbia University Medical Center, New York, NY; Albert Einstein College of Medicine, New York, NY; Mount Sinai School of Medicine, New York, NY.

OT3-01-04
TANIA: A Randomized Phase III Trial Evaluating Continued and Reintroduced Bevacizumab (BEV) in Patients Previously Treated with 1st-Line BEV for Locally Recurrent/Metastatic Breast Cancer (LR/mBC)
von Minckwitz G, Cortés J, Gilgrov J, Marschner NW, Puglisi F, Vedoljak E, Duenne A-A, Zielinski C. German Breast Group, LBFG Forschungs GmbH, Neu-Iseburg, Germany; Vall d'Hebron University Hospital, Barcelona, Spain; APHP Tenon-APREC, Paris, France; Outpatient Cancer Center, Freiburg, Germany; University Hospital of Udine, Udine, Italy; Centre of Oncology, Split, Croatia; F. Hoffmann-La Roche Ltd, Basel, Switzerland; Medical University of Vienna, Vienna, Austria.

OT3-01-05
PARP Inhibition after Preoperative Chemotherapy in Patients with Triple-Negative Breast Cancer (TNBC) or Known BRCA 1/2 Mutations: Hoosier Oncology Group BRE09-146
Miller KD, Perkins SM, Badve SS, Sledge GW, Schneider BP. Indiana University Melvin and Bren Simon Cancer Center.

OT3-01-06
A Phase 2 Study Investigating the Safety, Efficacy and Surrogate Biomarkers of Response of 5-Azaditidine (5-AZA) and Entinostat (MS-275) in Patients with Advanced Breast Cancer
Connolly RM, Jankowitz RC, Andreopoulou E, Alfred JB, Jeter SC, Zorzi J, Adam BM, Espinosa-Delgado I, Baylin SB, Zahnov CA, Ahuja N, Davidson NE, Stearns V. Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins University, Baltimore, MD; University of Pittsburgh Cancer Institute, Pittsburgh, PA; Albert Einstein College of Medicine, Montefiore Medical Center, New York, NY; Mayo Clinic, Rochester, MN; National Cancer Institute, Bethesda, MD.

OT3-01-07
The BEACON Study (BrEAsT Cancer Outcomes with NKTTR-102): A Phase 3 Open-Label, Randomized, Multicenter Study of NKTTR-102 Versus Treatment of Physician's Choice (TPC) in Patients (pts) with Locally Recurrent or Metastatic Breast Cancer (MBC) Previously Treated with an Anthracycline, a Taxane, and Capecitabine (ATC)
Avada A, Leung ACF, Zhao C, Hannah AL, Perez EA. Universite Libre de Bruxelles, Brussels, Belgium; Nektar Therapeutics, San Francisco, CA; Mayo Clinic, Jacksonville, FL.

OT3-01-08
Phase II Study of 5-1 Combined with Cisplatin in the First-Line Treatment of Triple Negative Breast Cancer
Fan Y, Xu Bt. Cancer Hospital & Institute,Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, China.

OT3-01-09
Phase 3 Trial Comparing Capecitabine in Combination with Sorafenib or Placebo for Treatment of Locally Advanced or Metastatic HER2-Negative Breast Cancer (RESILIENCE)
Baselga J, Schwartzberg LS, Petrenicci O, Shan M, Gradishar WJ. Massachusetts General Hospital Cancer Center; West Clinic; Bayer HealthCare Pharmaceuticals; Feinberg School of Medicine, Northwestern University.

OT3-01-11
A Randomized, Phase II Multicenter, Double-Blind, Placebo-Controlled Trial Evaluating MetMab and/or Bevacizumab in Combination with Weekly Paclitaxel in Patients with Metastatic Triple-Negative Breast Cancer
Daniel BR, Campone M, Dieras V, Envin T, Yu W, Paton VE, Xia Q, Peterson A. Chattanooga Oncology Hematology Associates - SCHR, Chattanooga, TN; Centre Rene Gauducheau, Nantes-Saint Herblain, France; Institute Curie, Paris, France; Florida Cancer Specialists, Englewood, FL; Genentech Inc, South San Francisco, CA.

OT3-01-12
Withdrawn

OT3-01-13
Phase One Trial of Combined Temsirolimus, Erlotinib, and Crizotinib in Advanced Solid Tumors

OT3-01-14
N0937: Phase II Trial of Brostallicin and Cisplatin in Patients with Metastatic Triple Positive Breast Cancer
Moreno-Aspitia A, Rowland KM, Liu H, Hillman DW, Stella PJ, Perez EA, Mayo Clinic, Jacksonville, FL; Mayo Clinic, Rochester, MN; Carle Cancer Center, Urbana, IL; St Joseph Mercy Cancer Center, Ypsilanti, MI.

OT3-01-15
Phase III Trial Evaluating the Addition of Bevacizumab to Endocrine Therapy as First-Line Treatment for Advanced Breast Cancer: The LEA Study
De la Haba-Rodriguez JR, von Minckwitz G, Martin M, Morales S, Crespo C, Guerrero A, Anton-Torres A, Gil M, Muñoz M, Carrasco E, Rodríguez-Martin C, Porras I, Akbas T, Schooneg E, Tio J, Mehta K, Loibl S, on Behalf of GEICAM and GBG. University Reina Sofia Hospital, Córdoba, Spain; German Breast Group, Neu-Isenberg, Germany; Universital Gregorio Marañon, Spain; Hospital Aramu de Vilanova de Llerda, Spain; Hospital Universitario Ramón y Cajal, Spain; Instituto Valenciano de Oncologia, Spain; Miguel Servet University Hospital, Spain; Institut Catala d'Oncologia, Spain; Provincial Hospital University Clinic, Barcelona, Spain; GEICAM Headquarters, Madrid, Spain; University Hospital, Essen, Germany; Praxis Dr Schooneg, Berlin, Germany; Universitätsklinikum Muener, Germany.

OT3-01-16
A Phase 2 Study of Ridaforolimus (RIDA) and Daltuzumab (DALO) in Estrusgon Receptor Positive (ER+) Breast Cancer
Lu BD, Blum JL, Cortes J, Rugo HS, Swanton C, Eaton L, Song Y, Zhang T, Ebbinghaus SW, Baselga J. Merck Research Laboratories, Kenilworth, NJ; Texas Oncology, Texas Oncology, Dallas, TX; Vally D'Hebron University Hospital, Barcelona, Spain; UCSF Helen Diller Family Comprehensive Cancer Center, San Francisco, CA; The Royal Marsden Hospital, Sutton, United Kingdom; Merck Research Laboratories, North Wales, PA; Merck Research Laboratories, Boston, MA; Massachusetts General Hospital, Boston, MA.

OT3-01-17
Randomized, Double-Blind, Placebo-Controlled Phase II Trial of Low-Dose Metronomic Cyclophosphamide Alone or in Combination with Velliparib (ABT-888) in Chemotherapy-Resistant ER and/or PR-Positive, HER2/neu-Negative Metastatic Breast Cancer: New York Cancer Consortium Trial P8853
Andreopoulou E, Chen AP, Zujewski JA, Kalinsky K, Vahdat L, Raptis G, Hershman D, Novic Y, Muggia F, Sparano J. Albert Einstein College of Medicine/Montefiore Medical Center, Bronx, NY; National Cancer Institute, Bethesda, MD; Columbia University Medical Center, New York, NY; Weill Cornell Medical College, New York, NY; Mount Sinai School of Medicine, New York, NY; New York University Langone Medical Center, New York, NY.

OT3-01-18
Combination Immunotherapy with Trastuzumab and the HER2 Vaccine E75 in Low and Intermediate HER2-Expressing Breast Cancer Patients To Prevent Recurrence
Sears AK, Clifton GT, Wreland TJ, Hale DF, Ponniah S, Mittendorf EA, Peoples GE. Brooke Army Medical Center, Ft. Sam Houston, TX; Uniformed Services University of the Health Sciences, USMCI, Bethesda, MD; UT M.D. Anderson Cancer Center, Houston, TX.
OT3-01-19  Phase II Study of Topical Imiquimod and Weekly Abraxane for the Treatment of Breast Cancer Cutaneous Metastases
Higgins DM, Childs J, Parker S, Dits ML, Salazar LG. University of Washington, Seattle, WA.

Other

OT3-02-01  A 1-Year Prospective Longitudinal Study of the Role of Psychosocial Factors in Adherence to Adjuvant Endocrine Therapy in Early Breast Cancer
Song X, Verma S, Dent S, Clemmons M, Graham N, Bedard M, Paquet L. The Ottawa Hospital Cancer Centre, Ottawa, ON, Canada; Carleton University, Ottawa, ON, Canada.

OT3-02-02  Yoga Compared to Exercise as a Therapeutic Intervention during (Neoadjuvant) Chemotherapy in Women with Stage IInd Breast Cancer
Wiedermann F, Bussing A, Halle M, Kiechle M, Kohls N, Ostermann T, Satelier D, Ertl J. Technische Universität München, Munich, Germany; Universität Witten/Herdecke, Herdecke, Germany; Praxis Gynäkologie Arabella, Munich, Germany; Ludwig-Maximilians-Universität München, Bad Tölz, Germany.

OT3-02-03  Patient Empowerment by Group Medical Consultations in the Follow-Up of Breast Cancer Survivors and Surveillance of Women with a BRCA Mutation
van Laarhoven HWM, Prins JB, Schlooiz MS, Besselink, Bügemann L, van Koolwijk MPA, Hoogerbrugge N, Visser A. Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands.

OT3-02-04  TBCRC 012: ABCDE, a Phase II Randomized Study of Adjuvant Bevacizumab, Metronomic Chemotherapy (CM), Diet and Exercise after Preoperative Chemotherapy for Breast Cancer
Mayer EL, Ligibel JA, Burstein HJ, Peppercorn JM, Miller KD, Carey LA, Dickler MN, Mayer IA, Foreur EA, Eng-Wong J, Pletcher PJ, Ryabin N, Gelman R, Wolff AC, Winer EP. Dana-Farber Cancer Institute, Boston, MA; Duke University Medical Center, Durham, NC; Indiana University Melvin and Bren Simon Cancer Center, Indianapolis, IN; University of North Carolina at Chapel Hill, Durham, NC; Memorial Sloan-Kettering Cancer Center, New York, NY; Vanderbilt University, Nashville, TN; University of Alabama, Birmingham, AL; Georgetown University Lombardi Comprehensive Cancer Center, Washington, DC; Hoosier Oncology Group, Indianapolis, IN; Johns Hopkins Kimmel Cancer Center, Baltimore, MD.

7:30 pm–10:00 pm
OPEN SATELLITE EVENT
Marriott Rivercenter, Grand Ballroom
Clinical Applications for Preventing and Managing Skeletal-Related Events in Breast Cancer: A Current Perspective

SUNDAY, DECEMBER 10, 2011

7:00 am–9:00 am
REGISTRATION
Bridge Hall

7:00 am
CONTINENTAL BREAKFAST
Exhibit Hall C

8:00 am–10:00 am
SPECIAL REPORT
Exhibit Hall D

10:00 am–12:00 pm
THE YEAR IN REVIEW
Exhibit Hall D
Moderator: C. Kent Osborne, MD
Baylor College of Medicine
Houston, TX

Advances in basic breast cancer research
Kornelia Polyak, MD, PhD
Dana-Farber Cancer Institute
Boston, MA

Translational breast cancer research
Andrea Richardson, MD, PhD
Dana-Farber Cancer Institute
Boston, MA

Early breast cancer
Nancy E. Davidson, MD
University of Pittsburgh Cancer Institute
Pittsburgh, PA

Challenges and success: Treatment of metastatic breast cancer 2011
Hope S. Rugo, MD
University of California, San Francisco
San Francisco, CA

12:00 pm
ADJOURNMENT
ES1-3  
**Treatment of Metastatic Breast Cancer – Breast Cancer as a Chronic Disease: Triple Negative Breast Cancer.**  
Stearns V. Sidney Kimmel Comprehensive Cancer Center, Baltimore, MD

Carcinomas of the breast that do not express the estrogen or progesterone receptors (ER/PR) and are not associated with overexpression or amplification of HER2/new have been designated triple negative breast cancer. These tumors represent approximately 15-20% of all breast cancers, but are more prevalent in younger women, in African Americans, and in BRCA1 mutation carriers. Molecular profiling data suggest that many of triple negative tumors, but not all, are basal-like. The basal-like tumors are more likely to be resistant to standard therapies resulting in disease recurrence and poor prognosis. It is likely that triple negative breast cancer will be further sub classified to several additional distinct subtypes. Regardless of additional sub classification of the entity, the only treatment currently available for women with triple negative breast cancer is chemotherapy-based. In this symposium, I will review current molecular classification, prognosis and predictive markers, treatment options, emerging approaches, and clinical trials.

ES2-2  
**Mouse Models of Basal-Like Breast Cancer.**  
Jonkers J. Netherlands Cancer Institute, Amsterdam, Netherlands

Mouse models of human cancer provide powerful in vivo tools to study the mechanisms underlying drug response and acquired resistance. Once these processes are understood in sufficient detail it may be possible to design (combination) therapies that not only cause complete remissions but also eliminate remnant cells that might elicit recurrent disease. Women carrying germline mutations in BRCA1 are strongly predisposed to developing basal-like breast cancers, which frequently contain TP53 mutations. To study the role of BRCA1 loss-of-function in development of basal-like breast cancer, we and others have established human tumor xenograft models for basal-like-breast cancer as well as genetically engineered mouse models (GEMMs) for BRCA1-mutated basal-like breast cancer based on tissue-specific deletion of p53 and Brca1 [1]. The mammary tumors that arise in these mouse models show strong similarity to basal-like breast cancer with respect to expression of basal cell markers, high tumor grade, and lack of expression of hormone receptors and HER2 [2]. In addition, the BRCA1-mutated basal-like tumors in our mouse models are characterized by a high degree of genomic instability and hypersensitivity to DNA-damaging agents due to loss of homology-directed double-strand break (DSB) repair [3].

We have successfully used the xenograft models and GEMMs of BRCA1-deficient basal-like breast cancer for preclinical evaluation of therapy response and elucidation of mechanisms of acquired drug resistance. BRCA1-deficient mammary tumors are highly sensitive to PARP inhibitors and platinum drugs, but none of these drugs is capable of causing tumor eradication and all tumors grow back after drug treatment. Using functional genetic screens and mouse genetics, we found that therapy response and resistance is affected by several factors, including drug efflux transporter activity [3,4], type of BRCA1 founder mutation and 53BP1 status [5]. The lack of tumor eradication prompted us to look for additional drugs targeting BRCA1-deficient tumors. Using a cell-based screening approach, we found that bifunctional alkylators such as nimustine may cause durable complete remission of BRCA1-deficient mouse mammary tumors [6], suggesting that BRCA1-mutated hereditary breast cancers and BRCA-like sporadic tumors may be eradicated by dose-intensive treatment with bifunctional alkylators. In support of this notion, patients with breast cancers that display a BRCA1-like profile of genomic aberrations show a high complete remission rate and long progression-free survival after treatment with high-dose alkylating chemotherapy [7].

**References**

ES2-3  
**Patient-Derived Xenograft Models for Preclinical Breast Cancer Research: Not Just “Basal” Anymore.**  
Lewis MT. Baylor College of Medicine, Houston, TX

Translational breast cancer research is hampered by difficulties in obtaining and studying primary human breast tissue, and by the lack of in vivo preclinical models that reflect the range of patient tumor biology accurately. In an effort to overcome these limitations, several groups have recently succeeded in propagating patient-derived breast cancer xenografts representing multiple breast cancer subtypes, including estrogen and progesterone receptor positive, and HER2 positive tumors. In our laboratory, we have developed a diverse set of human breast tumors grown as xenografts in the mammary fat pad of SCID/Beige and NOD/SCID/IL2γ-receptor null (NSG) mice, two relatively new immunocompromised mouse models, under various transplant conditions. In all cases, xenograft lines were established directly from breast cancer patient samples, without intervening culture in vitro, using the epithelium-free mammary fat pad as the transplantation site. Of the conditions tested, xenograft take rate was highest in the presence of a low-dose estradiol pellet without exogenous human fibroblasts. Thirty five stably transplantable xenograft lines representing 27 patients were established, using pre-treatment, mid-treatment, and/or post-treatment samples. Most patients yielding xenografts were “triple-negative” (ER-PR-HER2-) (n=21). However, we were able to establish lines from three ER-PR-HER2+ patients, one ER-PR+HER2-, one ER-PR+HER2-, and one “triple-positive” (ER-PR+HER2+) patients. Serially passaged xenografts show phenotypic consistency with the tumor of origin at the histopathology level, and remarkable stability across multiple transplant generations at both the genomic, transcriptomic, and proteomic levels. Of 27 lines evaluated fully, thirteen xenografts showed metastasis to the mouse lung. These models, in conjunction with others like them, thus serve as renewable, quality-controlled tissue resources, and are proving useful for preclinical evaluation of experimental therapeutics.

ES3-3  
**Adjuvant Therapy in Patients with a Borderline HER-2 Status.**  
Odamian C, Migliaccio I, Santarpia L, Di Leo A. Hospital of Prato, Istituto Toscano Tumori, Prato, Italy

Adjuvant trastuzumab is indicated in individuals with early breast cancer which has HER2 gene amplification and/or HER2 protein overexpression. In the pivotal phase III adjuvant trials which tested the addition of trastuzumab to chemotherapy, positive HER2 status was defined as strong and complete cell membrane staining by immunohistochemistry (IHC) of >10% of invasive tumor cells or gene
amplification determined by fluorescence in-situ hybridisation (FISH) ratio of HER2 gene copy number to chromosome 17 centromeres (CEP17) of >2.0. The decision to use trastuzumab is based on a binary categorization of HER2 as positive or negative. As such, the definition of HER2 positivity is critical, as it dictates who will or will not receive potentially efficacious treatment. The challenge in creating guidelines for anti-HER2 treatment is that an HER2 result is described as positive or negative, but HER2 exists as a continuum of gene copy number and protein expression. 

A source of uncertainty in the management of patients is the discordance between the diagnostic thresholds for HER2 adopted in the adjuvant trastuzumab trials and those specified in the subsequently published American Society of Clinical Oncology and the College of American Pathologists (ASCO-CAP) guidelines. According to ASCO-CAP, HER2 positivity is defined by uniform intense and complete membrane staining by IHC in ≥30% of cells or HER2/CEP17 FISH ratio≥2.2. The definition is stricter than thresholds applied in the adjuvant trials, identifying a narrower population as HER2 positive. 

A further source of uncertainty is discordance between IHC and FISH. There is generally high concordance between the two methods, as increased HER2 protein is generally attributable to HER2 amplification. Discordant results may occur if one assay is correct and the other is incorrect, due to pre-analytic, analytic, and/or post-analytic error. True discordance may be attributable to intra-tumoral heterogeneity or polysomy chromosome 17. Within a tumor, HER2 amplification and/or overexpression may be detected in discrete focal HER2 amplified clones (FHAC) or in individual cells diffusely scattered on a dominant background of HER2 negative/equivocal expression. FHAC have been reported in association with discordance between IHC and FISH. Cells with polysomy chromosome 17 have extra copies of the HER2 gene. In such cases, tumors may be IHC positive although the HER2:CEP17 FISH ratio is not elevated. Notably, available data are inconsistent and uncertainty remains as to whether polysomy 17 without HER2 amplification is associated with protein overexpression. During this session, clinical situations with borderline HER2-status will be presented and discussed. Biological heterogeneity within the same tumor, polysomy chromosome 17, and moderate HER2 positivity may contribute to determination of an uncertain HER2 status, making unclear the benefit of adjuvant trastuzumab in these cases. Clinical considerations highlighting pros/cons of adjuvant trastuzumab in these cases, as well as updated biological information and clinical perspectives, will be presented.

ES5-1
Breast Cancer Trials in Developing Countries – Opportunities and Challenges.
Gupta S, Badwe RA, Mittra I, Nag S, Dawood S, Munshi A. Tata Memorial Hospital, Mumbai, Maharashtra, India; Jehangir Hospital, Pune, India; Dubai Hospital, Dubai, United Arab Emirates

Breast cancer (BC) incidence is low, but increasing in the urban areas of many developing countries (DC) because of adoption of western lifestyle, including reproductive and fertility patterns. Because of large populations in DC, the absolute number of BC patients is high. Distinctive features of BC in DC include younger median age (population pyramid effect), advanced stage at presentation, absence of screen detected cancers, higher fraction of triple negative and lower fraction of hormone responsive tumors and higher mortality/incidence ratio. Several common themes emerge when considering the design/implementation of BC trials in DC:

1. Many DC have established population-based cancer registration process with good quality data on BC incidence. However data on patterns of care and long term outcome is sparse. This affects the accuracy of baseline assumptions while designing BC trials.
2. There is lack of unique identification numbers for citizens with linked national health/death registers in DC that creates difficulties in follow-up. Ensuring adequate follow-up in BC trials requires special efforts in DC.
3. There is deficient cooperative group trials culture in most DC with consequent inefficient recruitment, retention and follow-up in clinical trials, despite large absolute numbers of BC patients. Investment in creating such a culture is likely to be very productive in the long term.
4. Healthcare community, civil society and governments in DC are insufficiently sensitized to the importance of clinical trials as drivers of improvement in health delivery, in addition to providing solutions for topical problems in BC. Sustained effort in this area is likely to result in better acceptance of clinical trials.
5. There are cultural differences with western countries (paternalistic healthcare systems with passive acceptance of healthcare by consumers) that makes informed consent (IC) process difficult. The complexity of IC documents prepared by western researchers in collaborative projects, coupled with the desire for literal translation into local languages, makes the IC process daunting, even for experienced researchers.
6. Regulatory and ethical oversight of BC trials in DC is variable, ranging from sophisticated to rudimentary. Several tertiary care academic centers have well organized institutional review boards that ensure proper conduct of BC trials. Approval of international collaborative projects, especially those that involve transfer of biological material for translational science, is difficult in many countries.
7. Since BC patients in DC receive fewer lines of therapy as a routine standard, there are opportunities to research new interventions in less heavily treated patients.
8. There is opportunity to research technology that has not yet been widely adopted in DC (such as sentinel lymph node technique versus other forms of reduced axillary surgery, newer radiation delivery techniques) in randomized trials.
9. Testing neoadjuvant therapies in large tumors is very feasible in DC because of large numbers.

There are other research opportunities in DC that can be themes of collaborative research with developed countries. Simplicity of design and ease of implementation will facilitate such collaborations. These will be discussed in the final presentation.

ES5-3
Novel Approaches to Breast Cancer Management in Low-Income Countries.
Kerr DJ. Africa-Oxford Cancer Consortium

There is clear evidence that the global burden of cancer is increasingly affecting low and middle – income countries, effectively those nations who have least health infrastructure to deal with complex diseases like cancer. A new approach to cancer therapy is required that combines efficacy, reduced side effects, cost-effectiveness with a delivery mechanism that does not rely on access to distant and expensive regional cancer centres. To this end we propose to develop a combination oral chemotherapy formulation which is likely to offer a clinically useful advantage to patients suffering from breast cancer For almost half a century, systemic therapy of cancer
has been dominated by the use of cytotoxic chemotherapeutics. Most of these drugs are DNA damaging agents or microtubule inhibitors that are designed to inhibit or kill rapidly dividing cells, often administered in single doses or short courses of therapy at the highest doses possible, with prolonged breaks (generally of 2–3 weeks in duration) between successive cycles of therapy. A reappraisal of the optimal way of administering chemotherapy is underway. Instead of using short bursts of toxic chemotherapy interspersed with long breaks to allow recovery from the harmful side effects, there is now a shift in thinking towards the view that more chronic and decelerated schedules of drug administration using much smaller individual doses than the MTD may be more effective — not only in terms of reducing certain toxicities, but perhaps also improving anti-tumour efficacy with an additional anti-endothelial cell effect, so-called ‘metronomic’ chemotherapy. We propose development of a flat dose, combination, oral chemotherapy formulation which will contain the following classes of antineoplastic agents, which have been shown to have activity in breast cancer. Fluoropyrimidines eg Capecitabine; Alkylating Agents eg Cyclophosphamide; Antiemetics eg Metoclopramide; administered continuously at doses inducing Grade 1 toxicity.

The drug doses will be carefully selected to provide moderately effective cancer control with reduced toxicity profiles. The clinical development pathway will be conducted in India and Sub-Saharan Africa with a view to providing a managed package of care which will allow delivery of the MetroPill to women with breast cancer in rural and minor urban settings rather than the regional cancer centres. We will train a cohort of district general hospital doctors/nurses to monitor and deliver the MetroPill, supported by a teaching package app and mobile phone technology to provide symptom support for patients. A phase 1 feasibility and pharmacokinetic study will be performed in 20–30 patients followed by single arm Phase 2 studies (n=40 patients) in breast cancer patients, which will report response rates, progression free survival and quality of life as a prelude to phase 3 trials. We believe that this is the sort of lateral approach we must consider if we are to take elements of cancer therapy to those regions which lie beyond the shadow of conventional cancer centres.

ES6-1

**Oncofertility: Translation in Multiple Dimensions.**

Woodruff TK. Northwestern University

Breast cancer is a disease associated with aging. In the US, the median age of breast cancer diagnosis and death is 61 years and 68 years, respectively. The number of older adults in the US is rising, and the number of individuals age 65 and older is expected to double by the year 2030. This demographic shift will lead to an increase in the number of individuals diagnosed with breast cancer. It is estimated that from 2010 to 2030 there will be a 57% growth in the number of new cases of breast cancer in patients age 65 and older.1 A challenge in caring for older adults with breast cancer is that they have been historically underrepresented in clinical trials that set the standard for breast cancer care.2 However, a growing body of evidence-based literature can be utilized to inform and optimize the care of the older adults with breast cancer. This presentation will focus on the treatment of early stage breast cancer in older adults, including the risks and benefits of local and systemic therapies, as well as survivorship issues. Recently reported phase III studies have led to advances in the treatment of older adults with breast cancer. A phase III randomized study evaluated the role of radiation therapy following lumpectomy in patients age 70 and older with stage I hormone receptor (HR) positive breast cancer who received treatment with tamoxifen. Receipt of radiation therapy was associated with a decrease in the risk of locoregional recurrence; however, there was no difference in overall survival. The study results underscore the importance of competing comorbidity as a cause of death in older adults with early stage breast cancer.3 The role of axillary lymph node dissection was evaluated in a randomized study of women over age 60 with node-negative, HR positive breast cancer who received treatment with tamoxifen. Patients were randomized to breast surgery and axillary dissection, or breast surgery alone. There was no difference between disease-free and overall survival in the 2 study arms.4 A phase III randomized study evaluated the efficacy of standard adjuvant IV chemotherapy (doxorubicin and cyclophosphamide [AC] or cyclophosphamide, methotrexate, and 5-fluorouracil [CMF]) versus oral chemotherapy (capecitabine) in patients age 65 and older with stage I-III breast cancer. Those patients who received standard chemotherapy showed improvement in progression-free and overall survival compared with patients who received capecitabine. This study demonstrates that IV polychemotherapy regimens remain the standard of care for older adults who are prescribed adjuvant chemotherapy.5 These phase III studies advance our understanding in the care of older adults with breast cancer. Furthermore, they demonstrate the feasibility and importance of accruing older adults to phase III trials, which will improve our evidence-based care of this growing population.
References

ES8-1
Breast Reconstruction with Implants and Post-Mastectomy Irradiation: Algorithm and Outcomes.
Cordeiro PG. Memorial Sloan-Kettering Cancer Center, New York, NY
Breast reconstruction in the face of radiotherapy proposes a unique set of challenges to the plastic surgeon and often alters the algorithm of the reconstruction. The impact of radiation changes on the alloplastic breast reconstruction is unpredictable. The author will review current approaches to reconstruction of the patient if they are to undergo post-mastectomy radiation. The algorithm for reconstruction and outcomes after radiation will be presented.

ES8-3
Measuring Outcomes in Breast Reconstruction: The Patient Perspective.
Pusic A. Memorial Sloan-Kettering Cancer Center, New York, NY
In breast reconstruction, understanding patients’ perceptions of surgical results is of primary importance. As new reconstructive techniques continue to advance, surgeons and patients require high quality information on key outcomes such as patient satisfaction and quality of life. The need for meaningful data is further accentuated by concerns over healthcare expenditures and the increasing involvement of patients in their own surgical decision-making. Patient-reported outcome (PRO) measures are questionnaires specifically designed to quantify aspects of outcome such as patient satisfaction and health-related quality of life. When developed and validated according to internationally recognized standards, PRO measures can provide reliable and valid assessment of patient outcomes. Traditionally, PRO measures were used in clinical research alone; however, advances in psychometric methods have now facilitated the development of a new generation of PRO measures that are also useful in clinical care. This presentation will provide an overview of PRO research in breast reconstruction. The BREAST-Q, a new PRO measure for breast surgery patients, will be highlighted. Physicians will gain the necessary critical appraisal skills to interpret and apply evidence from PRO studies in their own clinical practice. Approaches to routine use of PRO measures in clinical care will also be described.

DL1-1
Adaptive Resistance to Targeted Therapies.
Brugge JS, Muranen T, Zoeller J, Worster D, Iwanicki M, Selfors L, Mills G. Harvard Medical School, Boston, MA; MD Anderson Cancer Center, Houston, TX
While therapies that target specific molecular alterations in human tumors are showing significant efficacy in the clinic, drug resistance remains a major obstacle. Studies from our laboratory and others have provided evidence that PI3K- and HER2- targeted therapies induce a program of molecular responses to drug treatment that significantly contributes to drug resistance. Interestingly, we have found that resistance in vitro and in mouse models is significantly enriched in cells attached to basement membrane. This resistance correlates with specific upregulation of the adaptive response in the matrix-attached cells. Importantly, inhibition of key components of the adaptive response, like BCL-2, abrogates the drug-induced adaptive resistance. We propose a model in which matrix-protection from targeted therapies allows survival of a subpopulation of tumor cells associated with matrix/basement membrane which expand and lead to more stable drug resistance.

PL1-1
How To Use Endocrine Therapy for Breast Cancer: Beyond the Basics.
Winer EP. Dana-Farber Cancer Institute
Over two thirds of all breast cancers are hormone receptor positive. The majority of these tumors have some degree of sensitivity to endocrine treatments. The approach to endocrine therapy has become more complex over the past decade, and there are more treatment choices than before. Not only are there choices about which endocrine therapy to use, but also important decisions about how long to continue treatment and how to sequence agents. It has become clear that the benefits and risks of endocrine therapy vary both from tumor to tumor and from person to person. The landscape will soon become even more complicated as targeted therapies are combined with endocrine therapy to prevent or reverse drug resistance. This lecture will focus on a number of key issues: 1) Can we improve upon a 5 year course of tamoxifen in premenopausal women? 2) Given the high risk of late recurrence in many patients with hormone receptor positive disease, which patients will benefit from extended adjuvant therapy and what should this therapy be? 3) Is the use of an aromatase inhibitor alone in postmenopausal women sufficient therapy for all breast cancer patients and how should we select individuals who should be considered for other approaches? 4) In 2011, are there patients with hormone receptor positive disease who should not receive adjuvant endocrine therapy? 5) What approaches can be used to circumvent resistance to hormonal therapy, and how quickly will they be incorporated into clinical practice? The talk will address the dilemmas that are most vexing for clinicians and have immediate relevance for clinical practice.

BS1-1
Stem Cells, Cancer, and Cancer Stem Cells.
Wahl GM. Salk Institute for Biological Studies
Significant advances in breast cancer prevention and treatment have come from strategies based on knowledge of mammary cell biology and the unique molecular fingerprints of individual tumors. The work I will present concerns the contribution of stem-like cells we and others have found in many breast cancers. It has been proposed that such cells may mediate cancer initiation, perpetuation, and
generation of tumor heterogeneity, drug resistance and metastasis. The problem of “intratumoral heterogeneity” is very important to understand, for it provides a plausible mechanism of resistance to targeted therapies and can contribute to metastasis, two critical problems to solve to increase survival and improve cure rates of breast cancer patients.

Cancer has been called a caricature of embryogenesis. However, we are not aware of studies to determine whether mammary stem cells arise during mammogenesis, or whether they are only found in the adult. If such cells do arise during development, do they exhibit similarities to the stem-like cells detected in breast cancers? We explored these questions by isolating stem cells from mice, and then studying archival tumor microarrays and human cell lines. We dissected each stage of mouse mammogenesis and determined that fetal mammary stem cells (fMaSC) are not measurable by transplantation analyses early in development. However, they increase in abundance dramatically in late development, at the time when the rudiment invades through the mesenchyme into the adjacent fat pad. The cell surface properties of these cells enabled us to purify them extensively, and determine the genes they express, as well as those expressed in the stroma with which they associate. We found that the fMaSCs have many similarities to aggressive breast cancers, including an impressive enrichment for the MaSC gene expression program in basal-like breast cancers. Interestingly, the associated fetal stromal (fSTR) cells have similarities to what have been termed “Cancer Stem Cells”, and we found the fSTR signature enriched in the rather rare Claudin-low and metaplastic breast cancers. We have also identified growth regulatory pathways that may provide therapeutic targets for breast cancers exhibiting an fMaSC-like gene expression signature.

Our studies establish significant links between the molecular pathways present in both the stem cell containing and the stromal associated compartments of the embryo. How stem-like cells arise in breast cancer, however, still remains an open question. An intriguing possibility is suggested by our observation that breast cancers encoding mutant p53 genes are also highly enriched for stem-like gene expression signatures. We propose that just as p53 deficiency increases the frequency of somatic cell reprogramming, p53 deficiency in breast cancer may enable cancer cells to revert to an embryonic stem-cell state that offers advantages for cancer cell survival, proliferation, and ability to invade adjacent tissue.

Funding: This work was funded by grants from the Breast Cancer Research Program of the Department of Defense, the Breast Cancer Research Foundation, Susan G. Komen for the Cure, and the G. Harold and Leila Y. Mathers Charitable Foundation.

BS1-2
Stem and Progenitor Cells and the Origins of Breast Cancer Heterogeneity.
Smalley MJ, Molyneux G, Melchor L. The Institute of Cancer Research, London, United Kingdom

Breast cancer heterogeneity results from different genetic lesions occurring in different stem or progenitor cells of origin. We previously demonstrated that deletion of the Brca1 tumour suppressor in basal stem cells or luminal estrogen receptor negative progenitors in the mouse mammary epithelium resulted in very different tumour phenotypes. Remarkably, the progenitor origin tumours most closely resembled human BRCA1 tumours. Having examined what occurs when the same genetic lesion is made is different cells of origin, we are now examining the results of making different lesions in the same cell. We have knocked out different combinations of Brca2, Pten and p53 in the luminal progenitors and carried out a comparative analysis of the resulting tumours. Confirming the hypothesis that tumour heterogeneity is dependent on both genetic lesion and cell of origin, widely differing tumour phenotypes were generated. Remarkably, one gene combination generated tumours in which the same cells expressed both basal and luminal cell lineage terminal differentiation markers. These results have important implications for our understanding of the origins of tumour cellular heterogeneity.

SR1-1
Breast Cancer and the Environment: A Life Course Approach – Report Release from an IOM Committee
Hertz-Picciotto I. University of California, Davis

In response to a request from Susan G. Komen for the Cure®, an Institute of Medicine (IOM) committee was convened to review and assess the strength of the science base regarding the relationship between breast cancer and the environment. As part of their charge, the committee members reviewed the evidentiary standards for identifying and measuring cancer risk factors and the methodological challenges involved in conducting research on breast cancer and the environment. They also considered the potential interaction between genetic and environmental risk factors. The committee’s report notes evidence-based actions that women could take to reduce their risk of breast cancer and offers recommendations for future research on breast cancer and the environment. The results of this IOM committee’s work will be presented.

SR1-2
Breast Cancer and the Environment: A Life Course Approach – Report Release from an IOM Committee
Hiatt RA. University of California, San Francisco, San Francisco, CA

In response to a request from Susan G. Komen for the Cure®, an Institute of Medicine (IOM) committee was convened to review and assess the strength of the science base regarding the relationship between breast cancer and the environment. As part of their charge, the committee members reviewed the evidentiary standards for identifying and measuring cancer risk factors and the methodological challenges involved in conducting research on breast cancer and the environment. They also considered the potential interaction between genetic and environmental risk factors. The committee’s report notes evidence-based actions that women could take to reduce their risk of breast cancer and offers recommendations for future research on breast cancer and the environment. The results of this IOM committee’s work will be presented.

BS2-2
Nuclear Receptor Coactivators: Physiology and Disease.
O’Malley BW. Baylor College of Medicine, Houston, TX

Nuclear receptors control gene expression by recruiting transcriptional coactivators (or corepressors). The coactivators are ‘master regulators’ that activate genes to control physiologies such as reproduction, growth and metabolism. Consequently, they are major players in the development of many inherited and acquired endocrine-related pathologies such as fertility/endometriosis, cancers, and disorders of carbohydrate, lipid and protein metabolism. The coactivator proteins provide important insights to polygenic diseases and represent a new class of drug targets for therapeutic interventions.
MS1-1
Imaging Cancer Invasion In Vivo: Mechanisms and Implications for Therapy.
Friedl P. Radboud University Nijmegen

The tumor microenvironment contributes to cancer invasion, growth and survival and thereby impacts tumor responses to therapy. Using infrared-excited multiphoton microscopy in orthotopic fibrosarcoma and melanoma xenografts, we here identify a novel radio- and chemoresistance niche consisting of invading tumor cell strands consisting of several hundred connected cells located within collagen-rich stroma nearby blood and lymph vessels. Despite normoxia, perivascular invasion strands were resistant to high-dose hypofractionated irradiation which otherwise was sufficient to induce regression of the tumor main mass. This invasion-associated chemo- and radioresistance was sensitive to the simultaneous inhibition of β1 and β3 integrins by RNA interference or combined anti-β1/αV integrin antibody treatment leading to proliferation arrest, anoikis induction and subtotal to complete regression of both tumor lesion and invasion strands. Thus, collective invasion is an important invasion mode in solid tumors into a microenvironmentally privileged perivascular survival niche which conveys radioresistance by integrin-dependent signals.

MS1-2
Tumor Entrained Neutrophils Inhibit Seeding in the Pre-Metastatic Lung.
Granot Z, Heneke E, Comen EA, King TA, Norton L, Benezra R. Memorial Sloan-Kettering Cancer Center

Primary tumors have been shown to prepare distal organs for later colonization of metastatic cells by stimulating organ-specific infiltration of bone marrow derived cells. We have recently demonstrated that neutrophils are mobilized by the primary tumor and accumulate in the lung prior to the arrival of metastatic cells in mouse models of breast cancer. Tumor-engraded neutrophils (TENs) inhibit metastatic seeding in the lungs by generating H2O2 and tumor secreeted CCL2 is both required and sufficient for optimal anti-metastatic entrainment of G-CSF-mobilized neutrophils. TENs are present in the peripheral blood of breast cancer patients prior to surgical resection but not in healthy individuals. Thus, while tumor-secreted factors contribute to tumor progression at the primary site, they concomitantly induce a neutrophil-mediated inhibitory process at the metastatic site.

MS1-3
Tumor Heterogeneity and Metastasis.
Ellis MJ, Perou C, Carey L, Mardis E. Washington University School of Medicine, St Louis, MO; UNC, Chapel Hill, NC

Massively parallel sequencing using Next Gen sequencing approaches have opened a new window into our understanding of the molecular architecture of breast cancer. A significant advantage of these technologies are that they are digital in nature because the measurement technology begins with single DNA molecules attached to a substrate. As a result, not only do these technologies provide an efficient means to identify somatic mutations, information is also provided on the frequency with which any given mutation is observed within the population of DNA molecules in the sample under analysis. The first and most striking feature of Next Gen analysis of breast cancer is the large numbers of somatic mutations that have been identified. Genome wide, thousands of mutations can be present in a single tumor. While this finding generates immense complexity from the perspective of defining biological drivers versus passengers, it does open up a new opportunity to study variations in mutation frequency. In a perfectly monoclonal tumor with a diploid genome and no contaminating normal cell DNA the mutation frequencies generated by digital sequencing will be 50% for a heterozygous mutation or 100% for homozygous mutations. Very few breast cancers subjected to whole genome sequencing exhibit this pattern, rather mutations occur with a wide variation of frequencies from the detection limit, around 5%, through to 100%. Furthermore mutation frequency clustering often occurs, a phenomenon best explained by the presence of a repertoire of mutations present in a founder clone that are present in all tumors, combined with additional less common mutations that represent a subdominant or minority population of cells that have arisen through additional clonal outgrowth.

In a multi-clonal model one of the most critical questions is “which clone determines the prognosis?”. One way to begin to answer this question is to sequence tumors before and after therapy and to compare primary tumors versus metastases. In our initial experience of a patient with a basal-like breast cancer in which a whole cancer genome was generated from her primary, her subsequent brain metastasis and a mouse xenograft generated from the breast primary, there was clear evidence of a shift in mutation frequency primary to metastasis, suggesting the brain metastasis arose from a minor sub-clone. The mutational profile of the xenograft more closely resembled the metastasis rather than the primary, suggesting the grafting process captured the metastatic clone (1).

In this invited lecture we will present an extension of our sequencing experiences with further human progenitor-mouse xenograft comparisons, comparisons between human primaries and paired metastases and also examples of ER+ breast cancer genomes obtained before and after neoadjuvant endocrine therapy. In all these experiments multiclonoality appears to be the rule rather than the exception. The clinical and biological implications of these findings will be discussed.


MS2-1
Early Life Exposures and Breast Cancer Risk: Preeclampsia and Puberty.
Forman MR. UT MD Anderson Cancer Center

Women diagnosed with preeclampsia (PE) in pregnancy have a 12-72% reduced risk of breast cancer compared to women with a normotensive pregnancy while her daughter from the index pregnancy also has a lower breast cancer risk than normotensive (NT) daughters. Why is this so? The preeclamptic (PE) pregnancy is highly androgenic with low levels of estrogens and insulin-like growth factor -1 levels. We followed a cohort of offspring of preeclamptic pregnancies, comparisons between human primaries and paired metastases and also examples of ER+ breast cancer genomes obtained before and after neoadjuvant endocrine therapy. In all these experiments multiclonoality appears to be the rule rather than the exception. The clinical and biological implications of these findings will be discussed.

and that age of greatest weight gain in infancy is associated with the odds of age at onset of puberty. Our findings and those of others in early life exposures and breast cancer risk provide clues to tailoring the effect of early exposures by maternal morbidity in pregnancy.

**MS2-2**

**Environmental Exposures, Epigenetics, and Epidemiology: Influence of Environmental Factors on Pubertal Maturation and Breast Cancer Etiology.**

*Huiatt RA. University of California, San Francisco, San Francisco, CA*

Although much of breast cancer etiology can be explained on the basis of reproductive and family histories, there remains concern among both the public and scientific communities about the possible role of environmental factors. Several well-established observations suggest a role of external environmental factors including the 5-fold international differences in incidence rates and the rapid inter-generational increases in incidence with migration from low to high incidence countries. Although epidemiologic studies in adult populations have generally failed to discover a strong role for environmental factors in breast cancer etiology, emerging data suggests that relevant exposures may have effects in early life during so-called “windows of susceptibility” and be missed by attempted measurement in adulthood. Exposures to ionizing radiation and to diethylstilbestrol during pubertal development and in utero, respectively, are two examples. Recently, more attention has been directed to other environmental exposures, especially endocrine disrupting chemicals (EDCs) during early development and the pubertal period in particular. Many EDCs can now be measured in biospecimens from young prepubertal girls and in pregnant women, and animal studies suggest that some of these may cause changes in reproductive systems.

Early menarche is a well-established risk factor for breast cancer and increases risk approximately 2-fold compared to later menarche. The average age at menarche has also dramatically decreased in developed countries over the last 100 years, likely as the result of environmental factors including diet. However, the relevant exposures and their mode and timing of action are not well understood. An on-going epidemiologic cohort study of 1239 pre-pubertal girls from diverse race/ethnic and socioeconomic backgrounds has documented still earlier ages of pubertal onset and is offering insights into the potential role of EDCs and other chemicals, diet, physical activity, psychosocial and genetic factors on pubertal onset, the age of menarche and other markers of early development. This research on early environmental exposures in humans is proving both exciting and challenging and requires consideration of the complex nature of breast cancer etiology.

**MS2-3**

**Breast Cancer and the Environment: Developmental Reprogramming of Cancer Susceptibility by Early Life Environmental Exposures.**

*Walker CL. Texas A&M Institute for Biosciences and Technology, Houston, TX*

In the last two decades it has become appreciated that adverse environmental exposures early in life can have a profound impact on susceptibility to adult diseases such as obesity, cardiovascular disease, diabetes, metabolic syndrome and cancer. The “developmental origins of health and disease” or DOHaD hypothesis, posits that environmental exposures during key periods of tissue development and organogenesis can “reprogram” the cell’s epigenome in a way that increases susceptibility to disease later in life. It is now clear that even brief environmental exposures can reprogram developing tissues and profoundly increase risk of developing cancer in adulthood decades after the exposures occurred. This developmental reprogramming has been best appreciated in the context of early life exposures to environmental agents such as xenoestrogens that mimic steroid hormones, with such exposures dramatically increasing susceptibility to hormone-dependent tumors of the breast and reproductive tract later in life. Recent advances in the field of environmental epigenomics have provided data that epigenetic alterations in both DNA methylation and histone methyl marks are induced during developmental reprogramming. In some tissues, xenoestrogens induce these epigenetic alterations via activation of non-genomic signaling, providing a direct link between xenoestrogen exposure and disruption of the cell’s epigenetic machinery. For example, xenoestrogen-induced activation of non-genomic PI3K/AKT signaling has been shown to target the histone methyltransferase EZH2, modulating the activity of this epigenetic “writer” and disrupting the proper “installation” of epigenetic histone methyl marks during development. Understanding the nature of the epigenetic reprogramming induced by early life exposures to xenoestrogens (and other environmental agents) has important implications for our understanding of breast cancer susceptibility across the life-course and for identifying effective strategies to prevent this disease.

**PL2-1**

**Macrophages as Novel Targets for Therapy in Breast Cancer.**

*Coussens LM. University of California San Francisco*

While BC has not historically been linked to underlying inflammation or infection, it exhibits tumor-associated inflammation marked by infiltration of innate and adaptive immune cells into developing tumors. In BC, macrophages are one of the most abundant innate immune cells present. BC-associated macrophages are regulated in part by colony stimulating factor 1 (CSF1), a key cytokine involved in monocyte/macrophage maturation, recruitment and activation, and its cognate receptor CSF1R. Macrophage presence in BC correlates with increased CSF1, increased vascular density, and worse clinical outcome. We reported that CD4+ T cells promote invasion and metastasis of mammary adenocarcinomas by directly regulating macrophage phenotype that in turn fosters invasive tumor growth, presence of circulating tumor cells and pulmonary metastasis. This preclinical data implied that women with BC heavily infiltrated by macrophages would have a worse clinical outcome as compared to tumors not heavily infiltrated with macrophages. We evaluated survival outcomes in 698 women with invasive BC treated with surgery alone and found that recurrence-free survival could be stratified based upon macrophage and T cell infiltration. Thus, we investigated CSF1 and CSF1R antagonists, in combination with standard-of-care chemotherapy (CTX) in mouse models of mammary carcinogenesis. We found that when macrophage infiltration in mammary adenocarcinomas was blocked, paclitaxel (PTX) chemosensitivity was increased, accompanied by development of productive anti-tumor immune responses and CD8+ cytotoxic T cell (CTL) infiltration. The combined effects of these changes were reduced primary tumor growth, 85% reduction in metastases and increased survival. In collaboration with clinical colleagues, we are currently evaluating the clinical benefit of macrophage modulation in preclinical models of BC to facilitate biomarker identification, and inform clinical trials of CTX in combination with macrophage-antagonists. Based on our preliminary data, we hypothesize that components of macrophage responses in BC can be identified to serve as biomarkers for risk stratification. And, that these components can be
effectively targeted for therapeutic intervention, resulting in reduced late-stage BC development and metastasis when combined with CTX. LMC acknowledges generous support from the NIH/NCI (R01CA130980, R01CA13256, R01CA140943, R01CA15531), the Department of Defense (W81XWH-09-1-0342, W81XWH-10-BCRP-EOHS-EXP) and the Susan G Komen Foundation (KG111084).

**BS3-1**

Extrinsic and Intrinsic Force Regulation of Breast Cancer Progression and Treatment.

**Weaver VM. University of California San Francisco, San Francisco, CA**

Breast tissue development and homeostasis are tightly regulated by mechanical cues. Alterations in tensional homeostasis characterize breast cancer. The challenge is to clarify how chemical and mechanical cues collaborate to regulate breast tissue function and to determine when and how these forces regulate breast cancer behavior. The Weaver group has been studying how cells in the breast sense and transduce mechanical cues to regulate their behavior and how altered mechanical force compromises breast tissue homeostasis to drive cancer (Dufort et al., Nature Mol Biol Rev 2011). Our work focuses on clarifying the role of force in normal breast behavior and in breast cancer initiation, transformation, metastasis and treatment response. We showed that extracellular matrix (ECM) stiffness, which is tightly controlled through matrix concentration, topology and posttranslational modifications, is a critical isometric force that modulates mammary gland morphogenesis and tumorigenesis. We demonstrated that the ECM associated with the mammary epithelium progressively stiffens as the tissue transforms and that breast cancer subtype is characterized by increasing ECM stiffness. Thus, normal human breast tissue is quite soft, the ECM associated with fibroproliferative lesions is twice as stiff, ER/PR+ve invasive breast cancers are 4 fold stiffer and triple negative breast lesions are the stiffest (6-10 times stiffer). We showed that a stiffened ECM modifies breast tissue behavior by promoting the assembly of integrin focal adhesions which potentiate growth factor receptor signaling and induce cytoskeletal remodeling and actomyosin contractility (Paszek et al., Cancer Cell 2005; Paszek et al., PLOS Computational 2009). We determined that ECM stiffness also potentiates the tumor promoting effect of oncogenes such as Ras, EGFR and ErbB2 by increasing the activity of ERK, wnt and PI3 kinase and inducing tissue fibrosis. Inducing ECM stiffening prevents breast tissue fibrosis and tumor progression and metastasis (Levental et al., CELL 2009). Importantly, the tissue response to force also depends upon the cellular mechanoresponsive machinery and we determined that oncogenic transformation and elevated chemokine and cytokine signaling induces aberrant force sensing and elevated breast tumor tension. Most notably we demonstrated that oncogenic signaling through Ras signaling to ERK induces ECM remodeling and stiffening which are required to stimulate wnt thereby driving tumor cell invasion to induce an epithelial to mesenchymal like transition that potentiates breast tumor aggression and metastasis. More recently we found that ECM stiffness dictates the efficacy of treatment response - with high ECM stiffness potentiating chemo, immune and radiation-induced tumor regression and apoptosis. The clinical relevance of these findings are now being investigated with collaborators at UCSF and Duke. (supported by DOD BCRP W81XWH-05-1-0330 and NCI U54CA143836-01, R01 CA138818-01A1, U01 CA151925-01, R01 CA085492, and R01 CA140663-01A2 to VMW, NIH grant 1U01ES009458-01 to ZW and NCI SPORE P50 CA058207 to VMW, CP, & SH).

**BS3-2**

Mammary Stroma as an NSAID Target; Implications for Pregnancy-Associated Breast Cancer.

**Schedin P, Lyons TR, O’Brien J, Callihan E, Russell T, Martinson H, Tan A-C, Hansen K, Keely PJ, Borges V. University of Colorado Denver Anschutz Medical Campus, Aurora, CO; University of Wisconsin, Madison, WI**

Utilizing a young women’s breast cancer cohort from the University of Colorado Cancer Center, we show that women diagnosed as late as five-to ten years postpartum have worse prognosis than nulliparous women or women diagnosed during pregnancy, and represent ~50% of all young women’s breast cancer patients. We propose that breast invasion following pregnancy accounts for this poor prognosis. Characterization of invasion identifies tissue remodeling programs that share similarities with microenvironments known to promote metastasis. Using SHG imaging, we find fibrillar collagen bundles with radially-aligned fibers similar to those observed in invasive tumors deposited in the involuting gland. By immunohistochemistry and FACS we find macrophages with an M2-polarization-like profile similar to tumor-associated macrophages at abundant levels during invasion. In three independent mouse models for post-partum breast cancer, we isolate postpartum mammary gland invasion as a driving force for cancer progression. Mammary tumors arising in the mouse involuting microenvironment express COX-2 and isolated tumor cells are motile and invasive in a collagen-1/COX-2 dependent manner. Targeting invasion-macrophages as likely mediators of tumor promotion was accomplished using a previously described mouse transgenic model. Macrophage depletion during involution had catastrophic effects on normal mammary gland involution. Conversely, inhibition of COX-2 with celecoxib, aspirin or ibuprofen did not interfere with postpartum lobular regression. COX-2 inhibition did decrease tumor growth, local tumor cell dispersion and lung metastasis. NSAID treatment also suppressed collagen and tenascin-C deposition in the involuting microenvironment, suggesting that modulation of extracellular matrix proteins may be a novel mechanism by which NSAIDs exhibit chemopreventive activity. Our studies indicate two distinct roles for COX-2 in the postpartum setting. COX-2 activity within the tumor cell is required for invasiveness and COX-2 activity in the host promotes collagen fibrillogenesis. Several correlative observations implicate the collagen/COX-2 pathway in postpartum breast cancer in women: involuting breast tissue has increased collagen with radially aligned fibers, analysis of 11 publicly available microarray data sets shows high COL1A and COX-2 independently correlate with decreased relapse free-survival in young breast cancer patients, and COX-2 protein is observed in DCIS lesions in postpartum cases at higher levels than nulliparous cases. In summary, our studies suggest further research into COX-2 inhibitor use might provide a novel strategy to improve the prognosis of young women should they be diagnosed with postpartum breast cancer. The question of whether an NSAID based intervention study could be aimed at recently pregnant women at high risk for breast cancer also remains to be determined, but is an extremely desirable objective given that the ~ 6 million pregnancies in the US per year. Supported by grants from DoD Synergistic Idea Awards BC060531 & BC10400/001, Komen Foundation KG090629, DoD Idea Award...
Inhibition of HIF-1 activity by genetic or pharmacologic strategies dramatically inhibits the metastasis of breast cancer cells to the lungs in orthotopic mouse models (2, 3). HIF-1 also plays critical roles in breast cancer metastasis. HIF-1 transactivation and reprograms glucose metabolism in cancer cells (1). Pyruvate kinase M2 (PKM2) coactivator function, reduces glucose uptake and lactate production, and PKM2 hydroxylation on proline-403/408. PHD3 knockdown inhibits PKM2 binding to HIF-1 subunit and promotes transactivation of HIF-1 target genes by enhancing HIF-1 binding and p300 recruitment to hypoxia response elements, whereas PKM1 fails to regulate HIF-1 activity. Interaction of PKM2 with prolyl hydroxylase 3 (PHD3) enhances PKM2 binding to HIF-1α and PKM2 coactivator function. Mass spectrometry and anti-hydroxyproline antibody assays demonstrate PKM2 hydroxylation on proline-403/408. PHD3 knockdown inhibits PKM2 coactivator function, reduces glucose uptake and lactate production, and increases O2 consumption in cancer cells. Thus, PKM2 participates in a positive feedback loop that promotes HIF-1 transactivation and reprograms glucose metabolism in cancer cells (1). HIF-1 also plays critical roles in breast cancer metastasis. HIF-1 controls metastatic niche formation by activating transcription of genes encoding lysyl oxidase (LOX) and LOX-like proteins 2 and 4, which remodel collagen in the lungs, thereby recruiting bone marrow-derived cells that establish a microenvironment suitable for colonization by breast cancer cells (2). HIF-1 also promotes the extravasation of circulating breast cancer cells in the lungs by activating transcription of the genes encoding L1CAM, which encodes a cell adhesion molecule that promotes the interaction of breast cancer cells with vascular endothelial cells (ECs), and angiopoietin-like 4, which encodes a secreted factor that inhibits EC-EC interaction (3). Inhibition of HIF-1 activity by genetic or pharmacologic strategies dramatically inhibits the metastasis of breast cancer cells to the lungs in orthotopic mouse models (2, 3).


**MS3-2**

**HIF-1, Metabolism, and Breast Cancer Metastasis.**

Luo W, Zhang H, Wong CC-L, Gilkes DM, Hu H, Semenza GL. The Johns Hopkins University School of Medicine, Baltimore, MD; University of Science and Technology of China, Hefei, Anhui, China

Increased glucose uptake and metabolism is a universal characteristic of advanced solid cancers. There are two well-established mechanisms underlying the reprogramming of tumor metabolism. First, intratumoral hypoxia induces the activity of the transcriptional activator hypoxia-inducible factor 1 (HIF-1) by inhibiting the O2-dependent prolyl and asparaginyl hydroxylases that inhibit HIF-1α stability and transactivation, respectively. Second, genetic alterations increase the activity of HIF-1, thereby increasing the expression of glucose transporters (GLUT1, GLUT3), glycolytic enzymes (ALDOA, ENO1, HK2, LDHA, PKM2), pH regulators (CAR9, NHE1, MCT4), and proteins that inhibit mitochondrial metabolism (BNIP3, PDK1). Metabolites, such as the glycolytic end-product lactate, also induce HIF-1 activity, thereby providing a signal to further increase glycolytic metabolism. Recently, we have identified a novel feed-forward mechanism by which glycolytic enzyme expression leads to increased HIF-1 transcriptional activity. Pyruvate kinase isoforms PKM1 and PKM2 are alternatively spliced products of the PKM2 gene. PKM2, but not PKM1, alters glucose metabolism in cancer cells and contributes to tumorigenesis by mechanisms that are not explained by its known biochemical activity. We show that PKM2 gene transcription is activated by HIF-1. PKM2 interacts directly with the HIF-1α subunit and promotes transactivation of HIF-1 target genes by enhancing HIF-1 binding and p300 recruitment to hypoxia response elements, whereas PKM1 fails to regulate HIF-1 activity. Interaction of PKM2 with prolyl hydroxylase 3 (PHD3) enhances PKM2 binding to HIF-1α and PKM2 coactivator function. Mass spectrometry and anti-hydroxyproline antibody assays demonstrate PKM2 hydroxylation on proline-403/408. PHD3 knockdown inhibits PKM2 coactivator function, reduces glucose uptake and lactate production, and increases O2 consumption in cancer cells. Thus, PKM2 participates in a positive feedback loop that promotes HIF-1 transactivation and reprograms glucose metabolism in cancer cells (1). HIF-1 also plays critical roles in breast cancer metastasis. HIF-1 controls metastatic niche formation by activating transcription of genes encoding lysyl oxidase (LOX) and LOX-like proteins 2 and 4, which remodel collagen in the lungs, thereby recruiting bone marrow-derived cells that establish a microenvironment suitable for colonization by breast cancer cells (2). HIF-1 also promotes the extravasation of circulating breast cancer cells in the lungs by activating transcription of the genes encoding L1CAM, which encodes a cell adhesion molecule that promotes the interaction of breast cancer cells with vascular endothelial cells (ECs), and angiopoietin-like 4, which encodes a secreted factor that inhibits EC-EC interaction (3). Inhibition of HIF-1 activity by genetic or pharmacologic strategies dramatically inhibits the metastasis of breast cancer cells to the lungs in orthotopic mouse models (2, 3).


**MS3-3**

**Energy Metabolism in Breast Cancer: Translational Science Insights Relevant to Effects of Diet, Exercise, and Metformin on Risk and Prognosis.**

Pollak M. McGill University and Jewish General Hospital

Energy metabolism is relevant to breast cancer at both the cellular and whole organism levels. Whole organism energy balance determines body mass, which has been associated with variations in both breast cancer risk and prognosis. Experimentally, breast carcinogenesis is facilitated by excess caloric intake and inhibited by caloric restriction. The simplistic notion that excess food intake provides additional energy to breast epithelial cells at risk for transformation or to breast cancers, leading to aggressive behavior, is not supported by experimental data. Rather, variations in energy balance have important influences on the hormonal and cytokine environment of the patient, and these influence carcinogenesis and tumor behaviour. Experimental models provide evidence that one such mediating hormone is insulin. Most breast cancers have insulin receptors. When mice with breast cancer are experimentally manipulated to have insulin deficiency (type I) diabetes, tumor growth rate is slowed (despite hyperglycemia). Conversely, when mice are provided with a “junk food” diet, insulin levels rise, tumor insulin receptor activation increases, and tumors grow more quickly. However, when breast cancers evolve to have activating mutations in signalling networks downstream of insulin receptors, they become more aggressive and unresponsive to variations in energy intake and insulin no longer influences their behavior.

There is retrospective pharmacoepidemiologic evidence for a substantial (~50%) reduction in breast cancer risk in type II (hyperinsulinemic) diabetic patients prescribed metformin. This has contributed to current interest in the hypothesis that metformin has uses in cancer prevention or treatment. Metformin acts to reduce cellular ATP production by inhibiting mitochondrial respiratory complex I. This results in activation of AMPK. In liver, this results in reduced gluconeogenesis, which reduces the hyperglycemia and hyperinsulinemia of type II diabetes. This systemic effect may reduce proliferation of the subset of neoplasms that are growth stimulated by insulin, but does not operate in the absence of baseline hyperinsulinemia. Other mechanisms of metformin action involve direct effects on at-risk or transformed cells. These mechanisms require adequate levels of the drug in the relevant cells, but metformin doses used in diabetes treatment may not achieve optimum concentrations in cancers, particularly those that lack the active transport molecules responsible for cellular metformin uptake. Overall, laboratory studies suggest that any benefits of metformin will not be homogeneous among a population of at-risk women in a prevention context, nor among breast cancer patients in a treatment context. The validation of candidate predictive biomarkers for metformin benefit, together with more detailed pharmokinetic data, may allow for optimized clinical trial design. Further research is also required to clarify if metformin should best be evaluated as a single agent or in combinations. Thus, metformin and derivatives can be regarded as lead compounds for optimization, and this line of research may lead to novel metabolic approaches to breast cancer prevention and treatment.
MS4-1
The Neoadjuvant Setting, HER2-Driven and Triple Negative Breast Cancer.
Carey LA. University of North Carolina

The benefits of neoadjuvant therapy include: tumor cytoreduction, minimizing surgical resection volume, conversion to breast conservation, and the opportunity to test new drugs and regimens because response in the breast is a surrogate for long-term survival. Off-trial, neoadjuvant regimens should mimic adjuvant regimens in drug, dose, and schedule; "tailored" approaches or treatment to best response have not been adequately studied. In HER2+ and triple negative (ER-, PR-, and HER2-negative, TNBC) BrCa, the backbone of neoadjuvant treatment is chemotherapy, with HER2-targeting added to the former. In hormone receptor-positive, HER2-negative BrCa, randomized clinical trials suggest similar efficacy of endocrine therapy and chemotherapy in response, however pathologic complete response (pCR) is rare.

HER2-targeting with the anti-HER2 antibody trastuzumab (H) added to chemotherapy for HER2+ BrCa augments pCR, with 1 year of H completed adjuvantly. Off-trial options include AC-doxetaxel (D) + H, AC-paclitaxel (T) + H, and D + carboplatin + H (DCH). It is likely that small, node-negative HER2+ BrCa are overtreated by these regimens; a challenge is to develop less chemotherapy-intensive regimens for the lower-risk setting. Several studies have examined other HER2-targeted drugs. In NeoALTO, HER2+ patients received either TH, T + HER1/HER2 inhibitor lapatinib (TL), or the combination TTHL. pCR was lower (and toxicity higher) in TL; THL had the highest pCR rate (47%). GeparQuinto compared EC-DH to EC-DL and also found lower pCR rate and higher toxicity in the L-containing arm. NeoSPHERE examined the anti-HER2 heterodimerization domain antibody pertuzumab (P), pCR rates were higher with combination DHP (46%) vs. DH (29%) and DP (24%). The all-biologic HP arm had a 17% pCR rate. In TBCRC006, HL alone resulted in a 28% pCR rate. These studies support combination HER2-targeting-based regimens in adjuvant trials.

TNBC lacks the known targetable molecules, ER, PR, or HER2, leaving chemotherapy the mainstay of treatment. Early TNBC is sensitive to conventional agents, and possesses one of the highest pCR rates to neoadjuvant anthracycline/taxane-based chemotherapy. The use of DNA-damaging drugs in TNBC is based on the association of Basal-like BrCa with BRCA1 germline mutations. Neoadjuvant studies are small and heterogeneous but supportive of a high pCR rate (>70%) to platinum in known carriers, however studies of sporadic TNBC are less clear. CALGB 40603, an ongoing randomized phase II study of taxane with or without carboplatin and with and without the anti-VEGF antibody bevacicuzumab (B), will provide direct evidence. Although lacking known targeted therapy, subset analysis of the HER2-negative component of GeparQuinto suggested that TNBC had higher pCR rates with EC-D + B (45%) compared with chemotherapy alone (36%). The biology and identification of novel targets in TNBC as well as optimization of the chemotherapy remain subjects of intense study.

Neoadjuvant therapy is an excellent approach for patients with clinical stage II-III in whom the nature of systemic therapy is clear. It also provides a rich laboratory for drugs, regimens, and the identification of predictive markers and subtype biology in a timely and efficient manner.

MS4-2
Preoperative Endocrine Therapy: New Approaches.
Smith I. The Royal Marsden Hospital, London, United Kingdom

Modern neoadjuvant endocrine therapy trials comparing aromatase inhibitors (AIs) with tamoxifen have shown that neoadjuvant AIs can downstage cancers and avoid mastectomy in around 50% of post menopausal women with large ER positive tumours, and are superior to tamoxifen in this respect.

The future potential of preoperative endocrine therapy is in the use of molecular biomarkers after starting treatment to predict outcome. This approach was pioneered in the IMPACT trial which showed that the reduction in tumour proliferation as measured by the biomarker Ki67 comparing neoadjuvant anastrozole v tamoxifen v. the combination. was significantly greater for anastrozole than for the other 2 arms, predicting the same long-term outcome benefit in the equivalent adjuvant ATAC trial. The change in Ki67 with treatment could be used to predict potential benefit with other novel endocrine therapy approaches. Recently a similar neoadjuvant trial comparing anastrozole v letrozole v exemestane has shown no significant difference in Ki67 suppression between arms, predicting no long-term difference in clinical outcome with these agents.

Around 15% of patients with good initial tumour Ki67 suppression after 2 weeks of preoperative endocrine therapy show recovery of Ki67 by 12 weeks, providing early biomarker evidence of treatment resistance. Clinical outcome in these patients appears correspondingly worse. This provides an opportunity for biomarker led studies of agents targeting both innate and acquired resistance to endocrine therapy, defined by 2 week and 12 week Ki67 levels,. One such preoperative endocrine therapy trial with Ki67 as primary endpoint has already been carried out: patients were started on 2 weeks of preoperative anastrozole and then randomised to the addition or not of the tyrosine kinase inhibitor, gefitinib (IRESSA). This novel pre-operative endocrine therapy approach also allows the study of changes in other biomarkers and in gene expression as a means of elucidating underlying mechanisms of resistance.

Finally the clinical potential of Ki67 after 2 weeks preoperative AI treatment is further demonstrated by the observation that higher 2 week values of Ki67 after treatment in the IMPACT trial predicted for a significantly worse recurrence-free survival. This raises the intriguing possibility that patients with ER positive breast cancer, including small cancers, could be treated with 2 weeks pre-operative endocrine therapy before surgery and Ki67 levels at surgery be used to predict long-term outcome for the individual patient and in particular to identify those patients with a good prognosis who would not require chemotherapy. This hypothesis is being tested in the UK POETIC (Peri-Operative Endocrine Therapy for Individualised Care) trial which has so far accrued around 2,000 patients out of an overall target of 4,000. A similar approach in which Ki67, pathologic stage and ER at the end of a course of neoadjuvant endocrine therapy are combined to produce a Preoperative Endocrine Prognostic Index (PEPI) is being used to identify patients with such a low risk of relapse that adjuvant chemotherapy may not be necessary.

MS4-3
Radiation for Patients Treated with Neoadjuvant Chemotherapy.
Buchholz TA. UT MD Anderson Cancer Center

The increased use of neoadjuvant systemic therapy for patients with stage II or stage III breast cancer has raised a number of important questions concerning local-regional treatments, particularly within the field of radiation oncology. Historically, the indications for radiation use after mastectomy and treatment of regional lymph node fields were
Axillary node status is one of the strongest prognostic factors in breast cancer patients. The presence of tumor in axillary nodes, and number of positive nodes, predicts risk of metastatic disease and long term survival. In the past, axillary dissection provided both accurate evaluation of nodal status and excellent local control. However, complications of axillary dissection, including lymphedema, pain, and impaired arm function, have led to keen interest in alternatives to axillary dissection for local control and assessment of prognosis.

Sentinel node biopsy provides a low-morbidity alternative to axillary dissection for patients with clinically negative nodes. It was recognized that breast lymphatics converge in the axilla, delivering fluid, travelling tumor cells and dye particles to a small number of “sentinel” nodes. Sentinel node biopsy techniques take advantage of this lymphatic anatomy using dye injection intraoperatively to permit identification and removal of only the most important axillary nodes – those most likely to contain metastases. A negative sentinel node provides accurate staging and reliably predicts a low axillary recurrence rate, eliminating the need for dissection. Sentinel node mapping also allows study of the relationship between the size of a nodal metastasis and impact on prognosis. Sentinel node mapping techniques, morbidity, and data on the reliability of sentinel node biopsy will be reviewed.

40-50% of patients with a positive sentinel node will have additional positive axillary nodes, indicating a need for additional axillary treatment for local control. Completion axillary dissection was initially used for local control and prognostic assessment in patients with a positive sentinel node. Recently, the ACOSSG Z0011 trial showed that patients with 1 or 2 positive sentinel nodes who receive standard whole breast irradiation without further axillary surgery have equivalent local control as patients having completion axillary dissection. Axillary recurrence rates in the Z0011 trial were <1% at 6.3 years follow-up in both the radiation alone and dissection arms. Changing axillary management algorithms resulting from these data will be discussed. Sentinel node biopsy can now replace axillary dissection for patients with negative nodes undergoing either lumpectomy or mastectomy. Sentinel node biopsy can also replace dissection for lumpectomy patients with 1-2 positive sentinel nodes where data on the precise number of additional positive axillary nodes is not needed for treatment decisions. At present, axillary dissection is still recommended for patients with palpable axillary nodes, 3 or more positive sentinel nodes, gross extranodal tumor, and positive sentinel nodes after neoadjuvant therapy, or for positive nodes in patients undergoing mastectomy. Trials of radiation instead of dissection in such patients are likely.

Management of the axilla will evolve further as options for assessment of tumor and host factors increase. Gene expression profiling may prove more important than primary tumor size and node status for predicting prognosis and guiding therapy – an approach already used in estrogen receptor positive tumors. Future options for incorporating axillary node status with tumor and host factors in breast cancer management will be considered.

**PL3-1**

Advances in Axillary Surgery: Sentinel Nodes and beyond.

**Smith BL. Massachusetts General Hospital**

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**SR2-1**


**Mardis ER. Washington University**

An important goal, that of characterizing the breadth and complexity of mutations in breast cancer, is being approached by The Cancer Genome Atlas in its next-generation sequencing and analysis of the exome and whole genome content of breast cancer samples. At present, we have produced, analyzed, and validated mutations by exome capture of matched tumor and normal genomic DNA across all breast cancer subtypes, using over 500 samples as our primary discovery set. The resulting information has been further analyzed by integration with other data types being generated in the project, including RNA-seq, methylation and SNP array data.
SR2-3
Integrative Genomic Analyses of Breast Cancer from The Cancer Genome Atlas (TCGA).
Creighton C. Baylor College of Medicine

Cancer is a disease characterized by genetic aberrations, which lead to widespread deregulation of cell signaling and gene transcription. Over ten years ago, gene transcription profiling of human cancers was found to distinguish distinct intrinsic molecular subtypes of the disease (luminal A/B, HER2, basal, normal-like). TCGA is a large-scale collaborative effort which seeks to comprehensively catalogue the molecular aberrations in various cancers, including breast cancer, at levels of somatic mutation, copy alteration, DNA promoter methylation, protein signaling, and gene and miRNA transcription. Having all of these molecular data on the same set of ~500 human breast tumors, allows us to construct a more complete molecular portrait of the intrinsic subtypes, along with their candidate molecular drivers. Among other things, integrative analysis of the p53 and PI3K pathways in breast cancer reveal differences in pathway alteration and pathway activity among the subtypes.

SR2-5
The DNA Copy Number Landscapes and Genomic Architecture; Relevance for Progression and Outcome.
Børresen-Dale A-L. Norwegian Radium Hospital, Oslo University Hospital, Oslo, Norway; Faculty of Medicine, University of Oslo, Oslo, Norway

Patterns of genomic aberrations have been defined that underlie specific expression subgroups of breast cancer (BC) that may infer paths of tumor progression and shed light on mechanisms involved. Classifications built on levels of genomic distortion have been shown to have prognostic value. We recently developed two platform-independent algorithms to explore genomic architectural distortion using aCGH data to measure whole-arm gains and losses [whole arm aberration index (WAAI)] and complex rearrangements [complex arm aberration index (CAAI)]. By applying CAAI and WAAI to data from 595 BC patients, we were able to separate the cases into eight subgroups with different distributions of genomic distortion. Within each subgroup data from expression analyses, sequencing and ploidy indicated that progression occurs along separate paths into more complex genotypes. Histological grade had prognostic impact only in the luminal-related groups, whereas the complexity identified by CAAI had an overall independent prognostic power. The DNA Copy Number Landscapes and Genomic Architecture; Relevance for Progression and Outcome.

SR2-6
Genome Data for the Masses: Presentation of TCGA and IGC Breast Tumor Data.
Haussler D. Howard Hughes Medical Institute

Large-scale cancer genomics projects will sequence tens of thousands of tumors in the next few years, along with matched normal tissue samples. Among these are the Cancer Genome Atlas (TCGA), Therapeutically Applicable Research to Generate Effective Treatments (TARGET), and the International Cancer Genomics Consortium (IGC). TCGA alone plans to analyze 500 clinically characterized samples from each of 20 different cancer types, detecting frequently mutated genes, common copy number variants, rearrangements, altered gene expression, and methylation changes. These data will provide an exceptional resource for identifying new diagnostic targets and predictors of response.

As a data analysis center and the primary sequence database for TCGA, we have built on technology developed for the UCSC Genome Browser to develop a cancer genome analysis pipeline and a cancer genomics browser (genome-cancer.ucsc.edu). These interpret cancer genomics data to aid in the identification of new targets. We reconstruct changes in tumor genomes and their expressed transcripts from tumor sequencing data (DNA, RNA, methylation) and use an approach based on factor graphs, called PARADIGM, to map multiple data types into a single coherent pathway model that includes thousands of genes and interactions for higher-level interpretation. By transforming raw genomic data to pathway activity levels, PARADIGM provides a comprehensible window into the data that can be coupled to predictors of response to improve accuracy. This paves the way to form hypotheses that clinical investigators can test in cell-line models and later explore in clinical trials.

Today's concurrent cancer genomic projects provide an exceptional opportunity to study the molecular nature of cancer on an enormous scale never before possible. A cancer genomics data repository that hosts multiple large projects and makes them accessible through a general cancer genomics browser (with effective data access control) will leverage each study and enable cross-tumor cancer research.
S1-1
Mehta RS, Barlow WE, Albain KS, Vandenbarg T, Dakhil SR, Tirumali NR, Lew DL, Hayes DF, Gralow JR, Livingston RB, Hortobagyi GN. University of California at Irvine, Chao Family CCC, Orange, CA; SWOG, Seattle, WA; Loyola University Chicago Stritch School of Medicine, Maywood, IL; London Regional Cancer Program, London, ON, Canada; Wichita CCOP, Wichita, KS; NW Permanente, Portland, OR; University of Michigan, Michigan; Seattle Cancer Care Alliance, Seattle, WA; Arizona Cancer Center, Tucson, AZ; University of Texas/MD Anderson Cancer Center, Houston, TX

Background: Anastrozole inhibits the aromatase enzyme-induced estrogen synthesis, and fulvestrant down-regulates estrogen receptors in hormone receptor-positive breast cancer. We hypothesized that the simultaneous disruption of the ligand-receptor axis with concurrent use of these agents may be potentially additive or synergistic in first-line therapy of hormone receptor-positive metastatic breast cancer in postmenopausal women.

Materials and Methods: A total of 707 patients were randomized to either 1 mg anastrozole P.O. daily (Arm 1) or to the combination of anastrozole and fulvestrant (Arm 2). Fulvestrant was given as an intramuscular injection as follows: loading dose of 500 mg on day 0, followed by 250 mg on days 14, 28 and 250 mg maintenance monthly thereafter. The primary endpoint was progression-free survival (PFS), with a power of 90% and one-sided alpha of 0.025 to detect an expected median PFS of 10 months in Arm 1 versus 13 months in Arm 2. Randomization was stratified by adjuvant tamoxifen use with a median age of 60 years in the fulvestrant arm (40%) and 50% in the anastrozole arm. Patients were encouraged to crossover to fulvestrant after progression on the anastrozole alone arm (40% did), if they were not candidates for immediate chemotherapy. Analysis of survival was by 2-sided stratified log-rank tests and Cox regression using intent-to-treat. Two interim analyses were performed with the final analysis using a 2-sided p-value of 0.04.

Results: There were 548 events (287 and 261 by arms, respectively) among 694 eligible patients (345 and 349, respectively). Overall, median PFS was 13.5 months for anastrozole and 15.0 months for the combination of fulvestrant and anastrozole (log-rank p = 0.0145; HR = 0.81 (95% CI 0.68-0.96)). In a subset analysis of tamoxifen-naive women (60%, n = 414), median PFS was 12.6 months and 17.0 months for Arms 1 and 2, respectively (log-rank p = 0.0069; HR = 0.74 (95% CI 0.60-0.92)), and 14.1 months and 13.5 months for Arms 1 and 2, respectively, in the 40% of tamoxifen-pretreated women (log-rank p = 0.56; HR = 0.93 (95% CI 0.71-1.20)). A trend for improved overall survival (OS) in the combination arm was seen (median OS was 42 and 48.6 months based on 152 and 137 deaths respectively, log-rank p = 0.094; HR = 0.82 (95% CI 0.65-1.03)). On the combination arm, there were 694 deaths related to the treatment: due to pulmonary embolism (2) or cerebrovascular ischemia (1); one Grade 4 thrombosis/embolism and one Grade 4 neutropenia and lymphopenia were reported. On the anastrozole arm, four patients experienced Grade 4 toxicities: thrombosis/embolism, arthralgia, thrombocytopenia, and dyspnea. In general, Grade 3 or higher toxicity was rare (12.7% vs. 14.5% for Arms 1 and 2, respectively) and did not differ significantly.

Discussion: The combination of anastrozole and fulvestrant was associated with improved PFS compared to anastrozole alone as well as a trend in OS despite substantial crossover, thus offering a new standard in the first-line treatment of hormone receptor-positive breast cancer in postmenopausal women, specifically in tamoxifen-naive patients. ClinicalTrials.gov:NCT00075764. Funding: NIH/NCI CA32102, CA38926 and AstraZeneca.

S1-2
Long-Term Follow-Up in ABCSG-12: Significantly Improved Overall Survival with Adjuvant Zoledronic Acid in Premenopausal Patients with Endocrine-Receptor–Positive Early Breast Cancer.
Gnant M, Milneritsch B, Luschin-Ehengreuth G, Stoeger H, Dubsky P, Jakess R, Singer C, Eidtmann H, Feil C, Eiermann W, Marth C, Greil R. Medical University of Vienna, Vienna, Austria; Paracelsus Medical University Salzburg, Salzburg, Austria; Medical University of Graz, Graz, Austria; University of Schleswig-Holstein, Kiel, Germany; Austrian Breast and Colorectal Cancer Study Group, Vienna, Austria; Red Cross Women’s Hospital, Munich, Germany; Medical University of Innsbruck, Innsbruck, Austria

Background: We have previously reported significantly improved disease-free survival (DFS) in premenopausal patients with endocrine-responsive early breast cancer receiving adjuvant zoledronic acid (ZOL) in ABCSG-12 (Gnant M, et al. NEJM. 2009;360:679-91). Other trials, such as ZO-FAST and the premenopausal (>5 yr) subset analysis of AZURE, have demonstrated similar anticancer effects with ZOL. Now with >6 yr of follow-up in ABCSG-12, we report an overall survival (OS) benefit and preplanned subgroup analyses that more precisely define interactions between the ZOL benefit and patient/tumor characteristics.

Methods: Premenopausal women with endocrine-receptor–positive early stage breast cancer (N = 1,803) were randomized to ovarian function suppression with goserelin (3.6 mg q28d) and tamoxifen (TAM; 20 mg/d) or anastrozole (ANA; 1 mg/d) ± ZOL (4 mg q6mo) for 3 yr. Endpoints included DFS and OS, both analyzed using log-rank test and Cox models.

Results: At median follow-up of 76 mo, patients receiving ZOL had a significant 27% reduction in the risk of DFS events (HR = 0.73; Cox P = .022) and a significant 41% reduction in the risk of death (HR = 0.59; Cox P = .027) vs no ZOL. Multivariate analyses showed a strong interaction between ZOL and patient age, but did not show any interactions between ZOL and ANA/TAM or any classic tumor parameter (eg, T, N, grade, ER). Among patients > 40 yr of age (n = 1,390) with presumed complete ovarian blockade, ZOL significantly reduced the risk of DFS events by 34% (HR = 0.66; Cox P = .014) and the risk of death by 49% (HR = 0.51; Cox P = .020); however, there were no significant DFS or OS benefits in patients <40 yr of age. Currently, all patients have completed 3 yr of ZOL and are in the follow-up phase with no reported cases of osteonecrosis of the jaw or renal failure. Additional analyses at a median follow-up of approximately 84 mo are planned for late 2011 and will be presented, providing additional insights into disease recurrence patterns with and without ZOL.

Conclusions: Long-term follow-up of ABCSG-12 (76 months) confirms and extends previous results seen at 48 mo and 62 mo follow-up, and suggests that anticancer benefits of adjuvant ZOL result in highly significant DFS and OS benefits mainly in patients with a low-estrogen environment (ie, ovarian suppression and age >40 yr). These results are consistent with the significant DFS and OS improvements seen in the postmenopausal (>5 yr) cohort of the AZURE trial, and suggest that both estrogen deprivation and reduction of bone-turnover–derived growth factors in the bone marrow microenvironment are needed for sufficient suppression of dormant micrometastases. Taken together with the previously demonstrated bone-protective effects of ZOL, these DFS and OS benefits strongly
suggest that adding ZOL to adjuvant endocrine therapy should be considered for premenopausal women with endocrine-receptor–positive early breast cancer.

S1-3
Long-Term Survival Outcomes among Postmenopausal Women with Hormone Receptor-Positive Early Breast Cancer Receiving Adjuvant Letrozole and Zoledronic Acid: 5-Year Follow-Up of ZO-FAST.

de Boer R, Bunded N, Eidmann H, Neven P, von Minckwitz G, Martin N, Modi A, Coleman R. Royal Melbourne Hospital, Victoria, Australia; University Hospital of South Manchester NHS Foundation Trust, Manchester, United Kingdom; University Frauenklinik, Kiel, Germany; UZ Gasthuisberg, Leuven, Belgium; German Breast Group, Frankfurt, Germany; Novartis Oncology, East Hanover, NJ; University of Sheffield, Sheffield, United Kingdom

Introduction: Recent clinical trials suggest potential anticancer activity for bisphosphonates combined with adjuvant endocrine therapy in patients with hormone receptor-positive (HR+) early breast cancer (EBC). Data from the interim analysis of AZURE suggest that the benefits of adding zoledronic acid (ZOL) may be greatest in patients with low estrogen levels (Coleman RE, et al. SABCS 2010). In ZO-FAST, we have previously demonstrated that adding ZOL to adjuvant therapy significantly improved bone mineral density (BMD) and prolonged disease-free survival (DFS) vs delayed ZOL (de Boer R, et al. SABCS 2010). We report here the effect of time since menopause at breast cancer diagnosis (ie, baseline menopausal status) on DFS benefits with ZOL.

Methods: Postmenopausal women with HR+ EBC receiving letrozole (LET; 2.5 mg qd × 5 yr) with a BMD T-score ≥-2 (N=1065) were randomized to ZOL (4 mg q6mo): immediate (IMZOL) or delayed (DZOL); initiated for postbaseline T-score <-2 or nontraumatic/asymptomatic fracture). Patients were followed for disease recurrence and overall survival (OS) for 5 years. Patients were eligible for the study if they had established menopause at the time of diagnosis, or if they became menopausal because of chemotheraphy or ovarian suppression (ie, recently postmenopausal). The effect of baseline menopausal status on DFS was examined in Cox regression analyses. Results: At 60 months’ follow-up in the overall population (N=1065), IMZOL significantly reduced the risk of a DFS event by 34% vs DZOL (hazard ratio [HR]=0.66; 95% confidence interval [CI], 0.44-0.97; P=0.034). In exploratory analyses of women who were postmenopausal for >5 years or >60 years old at study entry (n=670), IMZOL improved DFS (HR=0.63; 95% CI, 0.39-1.01; P=0.052) and significantly prolonged OS (HR=0.50; 95% CI, 0.27-0.92; P=0.022) vs DZOL. Additional subgroup analyses including patterns of breast cancer recurrence will be presented. During 5 years of treatment, osteonecrosis of the jaw (ONJ) was reported in 4/669 patients (0.6%) who received ZOL, and there was no increase in renal adverse events (AEs) in the ZOL-treated patients. Overall, AEs were consistent with the known safety profiles of both study drugs.

Conclusions: Long-term follow-up in ZO-FAST confirms the overall survival benefits of adding ZOL (4 mg q6mo) to adjuvant LET therapy for EBC. However, subset analyses suggest that women with established postmenopausal status may benefit from ZOL therapy more than others. These results are consistent with observations in the AZURE trial, and support potentially greater ZOL benefits in a low-estrogen environment. Additional studies are needed to fully define the patient populations most likely to benefit from adjuvant ZOL in this setting.

S1-4
Retrospective Analysis of Study EGF30008 by Mass-Spectrometry Based Serum Assay (VeriStrat®).

Roder J, Roder H, Hunsucker S, Grigorieva J, Ellis C, Florance A, Gagnon R, O’Rourke L, Johnston S, Biodesix Inc., Aurora, CO; GlaxoSmithKline, Collegeville, PA; Royal Marsden Hospital, London, United Kingdom

Background: In EGF30008 the addition of lapatinib (La) to first-line treatment with letrozole (Le) significantly improved progression-free survival (PFS) in patients (pts) with hormone receptor positive (HR+), HER2 positive (HER2+) metastatic breast cancer (hazard ratio (HR) = 0.71; P=0.019), but not in HR+, HER2 negative (HER2-) pts. This study assessed the ability of VeriStrat (VS), a mass-spectrometry blood-based pretreatment assay correlating with clinical outcome from EGFR-TKI therapy, to stratify pt outcome in EGF30008. The VS assay assigns VS Good (Good), VS Poor (Poor), or Indeterminate labels to a serum sample based on a specific 8-peak signature in the mass-spectra (Taguchi F et al., JNCI 2007).

Methods: Blinded to clinical data, pretreatment serum was analyzed using standard VS procedure. Statistical analyses were performed using Mantel-Haenszel and Cox proportional hazards methods; correlations were evaluated using Fisher’s exact and χ² tests.

Results: Of the 1286 pts randomized (HER2+=219; HER2-=952; HER2 unknown=115), 1163 pts had serum available; a VS label was assigned to 1046 pts (961 Good; 80 Poor; 5 Indeterminate); 117 were not evaluable (hemolyzed). In the overall population there was no significant difference in PFS between Good and Poor groups within the Le+La arm (p=0.53). In contrast, PFS of the Good group was longer than that of the Poor group within the Le only arm (HR=0.36, p<0.001) and comparable with that of Good pts within the Le+La arm (median PFS 10.8 mo vs 11.0 mo). An interaction test showed significantly different HRs for PFS in Good vs Poor between treatment arms (p=0.002).

In the HER2+ population both Good (n=169) and Poor (n=13) groups received significant PFS benefit from addition of La to Le: in Good HR=0.71, p=0.046, medians 3.0 mo (Le) vs 8.0 mo (Le+La); in Poor HR=0.17, p=0.02, medians 2.3 mo (Le) vs 8.6 mo (Le+La). In the HER2- population median PFS for the Poor group (n=58) increased from 3.1 to 11.0 mo (HR=0.57, p=0.068) with addition of La to Le, whereas the Good group (n=702) received no significant benefit, median 13.6 mo on Le and 13.8 mo on Le+La (PFS HR=0.85, p=0.09).

In multivariate analysis VS remained independently significant and predictive of differential treatment effect in the overall population. No significant correlation of VS classification with HER2 status (p=0.35) or prior adjuvant hormonal therapy (p=0.33) was found. VeriStrat was also not significantly correlated with baseline HER2 ECD levels (low <15 ng/ml vs. high ≥15 ng/ml, p=0.23, or ER expression (H-score:lower quartile:<160 vs. rest), p=0.18.

Conclusions: In the overall population VeriStrat predicted PFS in Le alone but not in the Le+La combination, with a significant differential treatment effect. VeriStrat identified a subset of pts with inferior PFS on Le therapy. Adding La to Le improved outcome in HER2+ pts, and interestingly suggested an improvement in the VS Poor subset of HER2- pts. HER2- VS Good pts do not gain benefit with the addition of La. No correlation between VS and investigated predictors of benefit from the addition of La to Le in HER2- pts was observed. If these results can be validated in a prospective study, the addition of La to Le may be a potential treatment option for HER2- HR+ VS poor pts.
**S1-5**

Modulation of Cancer and Stem Cell Biomarkers by the Notch Inhibitor MK-0752 Added to Endocrine Therapy for Early Stage ER+ Breast Cancer.

Albain KS, Czerlanius C, Zlobin A, Covington KR, Rajan P, Godellas C, Bova D, Lo SS, Robinson P, Sarker S, Gaynor ER, Cooper R, Aranha G, Czaplicki K, Busby B, Rizzo P, Demuth T, Stiff P, Fuqua SAW, Miele L. Loyola University Chicago Cardinal Bernardin Cancer Center; Maywood, IL.; Baylor Breast Center, Houston, TX; Merck Oncology, North Wales, PA; University of Mississippi Cancer Institute, Jackson, MS

**Background:** New strategies to enhance endocrine therapy (ET) efficacy and/or overcome resistance by targeting key survival pathways are needed. Preclinical data indicate that unwanted effects of ET include reactivation of the Notch pathway, critical for breast tumor initiating (stem) cells. Notch inhibition with gamma secretase inhibitors (GSI) enhances tamoxifen (tam) efficacy in xenografts, but impact of GSI+ET in human breast cancer (BC) is unknown. Our objective was to add short exposure of the GSI MK-0752 to ongoing tam or letrozole (let) in the presurgical window to assess feasibility, safety and biomarker/pathway impact in a 20-patient (pt) pilot study (ClinTrials.gov NCT00756717). We previously evaluated several biomarkers in the first cohort, which showed promise with Notch and proliferation inhibition. We present new results adding the final cohort, plus additional biomarkers and microarray analyses.

**Methods:** Pts with early stage ER+ BC received 25 days (d) of ET. MK-0752 was added d15 (350 mg PO 3d on, 4d off, 3d on) with definitive surgery d25. Core biopsies were done at baseline, d14 and d25, with qRT-PCR for Notch-related and other genes critical to stem cell renewal/proliferation. Gene expression levels after GSI (d25) vs ET alone (d14) were analyzed and d25 changes in all pts combined for each gene were compared. Microarray expression estimates and modeling were performed using dChip and Red-R, generating gene-wise comparisons using Limma. Probes were defined as significantly regulated by paired t tests if p ≤ 0.001 for the comparisons of baseline to tam/let and tam/let to tam/let+GSI. Data were exploratory so all probe data were included in the modeling, and no corrections for multiple comparisons were used. Differentially expressed genes were submitted to DAVID for pathway analysis.

**Results:** Of 22 pts accrued, 20 (11 tam, 9 let) were evaluable, meeting accrual goals (2 withdrew before MK-0752); 19 completed therapy to date. Toxicity was minimal. Significant (p<.05) changes in mRNA levels after GSI+ET vs ET in 17 pts (3 in progress) were down-regulation of Notch4 in 13; Ki67, 13; Notch1, 12; RUNX1 (stem cell transcription factor), 13; ADAM19 (disintegrin/metalloproteinase), 12; MMP7 (Wnt target), 11; CCND1 (cyclin D1), 10; and up-regulation of NOXA (pro-apoptotic BH3-only gene), 13. Microarray analyses (10 completed, remainder underway) defined as significantly regulated by paired t tests if p ≤ 0.001 for the comparisons of baseline to tam/let and tam/let to tam/let+GSI. Data were exploratory so all probe data were included in the modeling, and no corrections for multiple comparisons were used. Differentially expressed genes were submitted to DAVID for pathway analysis.

**Conclusions:** Short exposure of MK-0752 added to ET was feasible, well tolerated, and resulted in significant biomarker response in all tumors. MK-0752 favorably modulated proliferation, apoptosis, stem cell and metastasis-related targets, and impacted critical cancer pathways. This suggests potential roles for MK-0752 in optimizing endocrine therapy and overcoming endocrine resistance.

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**S1-6**

Characterization of Breast Cancer Distant Metastasis Based on Outcome over Time Using a Gene Expression Profiling Approach and Identification of Pathway Activities of Late Relapse.


**Background**

Previous reports have described the use of microarrays to assess the molecular classification of human breast cancers and defined new subgroups based on gene expression that are relevant to patient management through their ability to predict metastatic relapse and survival relapse. However, different mechanisms may be associated with the development of early and late distant metastases. With the hypothesis that tumors may lead to early or distant metastases based on their intrinsic biological initial features, we aimed at defining molecular profiles for several subgroups of patients based on their outcome over time.

**Material and methods**

Breast primary tumors were selected from retrospective series of patients with frozen material available. These series include patients of all ages, LN- and LN+; Estrogen or Progesteron-receptor positive, Her2-negative, no adjuvant treatment, with a follow-up of more than 10 years (y) for the control group or distant metastatic relapse as first event (DM) for the study group (n=144). Patients tumors were classified in 4 groups: no relapse at 10 y (M0), DM before 3 y (M0-3, n=30), DM between 3 and 7 y (M3-7), DM after 7 y (M7+). Samples were collected in 2 different institutions (NKI series for identifying the signature and IGR series for validation).

Gene expression analysis of breast tumor samples was performed using custom-made Agilent 44K high-density microarrays and hybridized against the MammaPrint® reference pool (MRP). Tumors were also assessed for their MammaPrint® status, wound-healing signature status and their intrinsic subtypes based on the Blueprint® signature. Moreover, we identified the pathway-level activities of the patient groups using PARADIGM.

**Results and Discussion**

For the NKI series, A subset of 144 samples was included based on the selection criteria: 57 M0, 31 M0-3, 25 M3-7, 31 M7+. None of the 3 previously mentioned signatures correctly identified M0 vs. M7+ patients. In order to identify a predictive signature of late relapse (after 7y) we considered M0 and M7+ MammaPrint-Low Risk patients and we split them in a training (n=41) and in a test (n=23) sets. A 73-gene signature was able to classify M7+ patients with 75% of sensitivity and 66% of specificity on the test set. DM after 7yr showed significant activation of pathway related to inflammatory response and angiogenesis. Detailed results and validation results on the independent IGR series will be presented at the meeting.
S1-7
Molecular Tumor Characteristics Influence Adjuvant Endocrine Treatment Outcome.
Bianchini G, Pusztai L, Iwamoto T, Kelly CM, Zambetti M, Fasolo A, Del Conte G, Santarpia L, Symmans WF, Gianni L. San Raffaele Cancer Center, Milan, Italy; University of Texas, MD Anderson Cancer Center, Houston; Mater Misericordiae University Hospital, Dublin, Ireland; Hospital of Prato and Istituto Toscana Tumori, Prato, Italy.

Background
Used upfront or after 2 to 3 years (yrs) of tamoxifen (TAM) or as extended treatment after 5 yrs of TAM, aromatase inhibitors (AIs) are associated with less recurrences than with 5 yrs of TAM. However, up front AIs were not superior to TAM→AI sequence in node negative (N-) tumors (TEAM study, SABCS 2009; BIG1-98 trial, NEJM 2009). The observation is consistent with the hypothesis that early relapses may be more frequently due to intrinsic endocrine resistance irrespective of the use of TAM or AIs whereas late relapses depend on acquired resistance.

Methods
Affymetrix HGU133A-based gene expression profiles from two adjuvant datasets (n=556) of patients treated with 5 yrs TAM were evaluated and included 285 N-, 247 N+ and 24 Nx estrogen receptor-positive cases. A proliferation score based on the expression of 12 mitotic kinases (MKS) (Bianchini, Cancer Res 2010) and a 4-gene estrogen-related score (ERS) adopted from the Oncotype DX Recurrence Score were assessed. Median cut-off points were used for both biomarkers. Pattern of relapse according to marker status were evaluated in three distinct time cohorts (0-2.5 yrs, 2.5-5 yrs, 5-10 yrs). Outcome was assessed according to distant relapse rates.

Results
A violation of the proportional hazards assumption was found for both scores indicating time-dependent effects. The table shows distant relapse rates according to biomarker group within each time cohort.

<table>
<thead>
<tr>
<th>Biomarkers group</th>
<th>No. pts</th>
<th>% Relapse</th>
<th>p</th>
<th>% Relapse</th>
<th>p</th>
<th>% Relapse</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>lowMKS/lowERS</td>
<td>162</td>
<td>1.3</td>
<td>0.111</td>
<td>2.7</td>
<td>0.139</td>
<td>14.4</td>
<td>0.006</td>
</tr>
<tr>
<td>highMKS/lowERS</td>
<td>116</td>
<td>4.4</td>
<td>8.7</td>
<td>17.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>lowMKS/highERS</td>
<td>116</td>
<td>1.8</td>
<td>&lt;0.001</td>
<td>13.1</td>
<td>0.634</td>
<td>27.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>highMKS/lowERS</td>
<td>162</td>
<td>15.8</td>
<td>15.8</td>
<td>16.0</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Among low proliferation tumors (lowMKS), the highERS group had low and steadily increasing recurrence rates, while the lowERS group showed a higher risk of relapse that increased continuously over the time. Among the highly proliferative tumors (highMKS), the high endocrine-sensitive group (highERS) had a lower risk of relapse within the 0-2.5 yrs compared to lowERS cancers. The difference is not persisting at the longer follow up of 2.5-5 yrs, and at 5-10 yrs the highERS group has higher risk of relapse. The pattern of relapse was similar for N- and N+ cancers. However, in the 0-2.5 yrs interval all recurrences occurred in the highMKS/lowERS among N- cancers, while only 57% of recurrences did occur in N+ cases in the same biomarkers group (p=0.012).

Discussion
Highly proliferative, high endocrine-sensitive (highERS) cancers are at the greatest risk of late relapse and extended endocrine therapy beyond 5 yrs may be useful. Low proliferation, low ERS cancers also remain at substantial risk for late recurrences. Between 0 to 2.5 yrs all recurrences in N- tumors and close to 60% in N+ were in high proliferative/low endocrine-sensitive tumors. This difference could explain the beneficial trend for up-front AIs vs. sequential TAM→AIs in the N+, but not in N- subgroups of the TEAM and BIG1-98 trials. It also is consistent with the hypothesis that the highly proliferative low ERS group includes tumors intrinsically resistant to both AIs and TAM. Risk stratification by these two simple metrics may improve the readout of future adjuvant clinical trials with endocrine drugs, and may influence their design.

S1-8
Molecular Signaling Distinguishes Early ER Positive Breast Cancer Recurrences Despite Adjuvant Tamoxifen.

Background: Unlike recurrences with other therapies, ER+ breast cancers (BC) can recur ≥10 yrs after an apparent successful period of adjuvant endocrine therapy. The molecular basis for this pattern of resistance is unresolved. We addressed the hypothesis that early recurrences during tamoxifen (TAM) treatment exhibit different biological characteristics than those that recur years later by assessing for variation in their respective transcriptionomes.

Methods: Appropriate tumors were identified from a set (BC030280) of snap-frozen tumor biopsies collected in Edinburgh from subjects with stage I-III BC before starting TAM (no chemotherapy); all had ≥10 yrs follow-up. Using rigorous standard operating procedures, high quality total RNA was extracted from samples with ≥50% malignant epithelium and arrayed on Affymetrix U133 Plus 2.0 GeneChips. Raw data were normalized using PLIER and analyzed with an adapted validated training and internal cross-validation workflow to avoid gene selection bias. A published dataset (Loi) was used for independent classifier validation that best fit our criteria for sample size, treatment (TAM only), data quality, and recurrence distribution. Early (E) vs late (L) recurrences were defined as distant relapse ≤3 vs ≥10 yrs from diagnosis. To explore putative mechanistic associations driving the transcriptome differences, a novel computational procedure was developed to integrate gene expression data with protein-protein interaction (PPI) data and create a statistical network model of the signaling. Metropolis sampling, a Markov Chain Monte Carlo method that can be implemented as a modified random walk procedure, identified ER network topology represented by the genes (nodes) and their predicted interconnections (edges).

Results: A support vector machine with recursive feature elimination was used for the binary classification tasks on the BC030280 dataset. The optimized classifier for E vs L recurrence was independently validated on the Loi dataset with high levels of accuracy, specificity, and sensitivity.

<table>
<thead>
<tr>
<th>E vs L (%)</th>
<th>accuracy</th>
<th>specificity</th>
<th>sensitivity</th>
<th>AUC</th>
<th>PPV</th>
<th>NPV</th>
<th>fDR</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>BC030280</td>
<td>24 vs 15</td>
<td>0.99</td>
<td>0.99</td>
<td>0.99</td>
<td>0.99</td>
<td>0.00005</td>
<td>0.00004</td>
<td></td>
</tr>
<tr>
<td>Loi</td>
<td>12 vs 19</td>
<td>0.77</td>
<td>0.83</td>
<td>0.74</td>
<td>0.81</td>
<td>0.67</td>
<td>0.01</td>
<td>0.0004</td>
</tr>
</tbody>
</table>

Classifier validation is supported by the respective survival curves (not shown). Gene set enrichment analysis reveals the top PPIs are primarily related to apoptosis (23/50; p=2.9e-13) and proliferation (14/50; p=6.8e-5). Substantial overlap of the network features and topology was seen between datasets. Specifically, increased relative expression of ESR1, ESR2, EGFR, BCL2, and AR was seen in L vs E recurrent tumors, and increased expression of CALM1, CALM2, SRC, CDK1, and MAPK1 was seen in E vs L recurrent tumors. Several hubs (nodes with ≥5 edges) independently predicted for recurrence in additional public datasets of ER+ BC.

Discussion: Our work provides clear evidence that robust molecular differences exist between ER+ BC that recur early vs. much later despite adjuvant TAM. Exploiting these differences will improve...
our understanding of involved signaling pathways, allow for the reliable prediction of early treatment failure, and guide use of novel therapeutics specifically directed at preventing E vs L recurrences on endocrine therapy.

S2-1
Partial Breast Brachytherapy Is Associated with Inferior Effectiveness and Increased Toxicity Compared with Whole Breast Irradiation in Older Patients.

Smith GL, Xu Y, Buchholz TA, Giordano SH, Smith BD. University of Texas M.D. Anderson Cancer Center, Houston, TX

Background: Accelerated partial breast brachytherapy (APBI-brachy) is an increasingly popular radiation treatment for older patients diagnosed with early stage breast cancer. Despite growing utilization, there is a lack of population-based cohort studies as well as randomized phase III data to compare its effectiveness and toxicity profile with standard whole breast irradiation (WBI). The purpose of this study was to provide the first nationally comprehensive comparison of effectiveness and toxicity outcomes in older Medicare patients treated with APBI-brachy versus WBI.

Methods: Medicare billing claims identified beneficiaries age ≥66 with incident invasive breast cancers diagnosed between 2000 and 2007 and treated with conservative surgery followed by APBI-brachy alone versus WBI. Cumulative incidence of subsequent mastectomy (a validated surrogate for local failure) was compared between the two treatment groups using the log-rank test. Adjusted risk of subsequent mastectomy was determined using a multivariate Cox proportional hazards model including demographic, socioeconomic, and clinical covariates. Risks of acute complications (hospitalization or infection within 120 days of radiation), were compared using the chi-square test. Adjusted odds of acute complications were determined using multivariate logistic models including covariates. Cumulative incidences of long-term toxicities (rib fracture, fat necrosis, breast pain, and pneumonitis) were compared using the log-rank test.

Results: In 130,535 women, use of APBI-brachy increased over time from <1% of patients treated in 2000 to 13% of patients in 2007 (P<0.001 for trend). Patients treated with APBI-brachy were less likely to have axillary lymph node involvement or to have received chemotherapy, and were more likely to be older, White, and have comorbid illness. At 5 years, the cumulative incidence of subsequent mastectomy was significantly higher in patients treated with APBI-brachy (4.0% in APBI-brachy vs. 2.2% in WBI, P<0.001). On multivariate analysis, there was a two-fold increased risk for subsequent mastectomy in patients treated with APBI-brachy (HR= 2.14; 95% CI 1.83-2.52, P<0.001). APBI-brachy was also associated with more acute complications, including a higher risk of hospitalization (9.6% vs. 5.7%; P<0.001) (Adjusted OR= 1.71; 1.58-1.86); and infection (8.1% vs. 4.5%; P<0.001) (Adjusted OR= 1.85; 1.69-2.02; P<0.001). APBI-brachy was also associated with higher 5-year cumulative incidence of rib fracture (4.2% vs. 3.6% in WBI), fat necrosis (9.1% vs. 3.7%), and breast pain (14.9% vs. 11.7%) (P<0.001 for all comparisons), but a lower incidence of pneumonitis (0.1% vs. 0.8%, P<0.001).

Discussion: APBI-brachy was associated with inferior effectiveness as well as increased acute and late toxicities compared with WBI in this cohort of older breast cancer patients. These data underscore the importance of awaiting mature results of randomized trials designed to prospectively compare these treatments before employing widespread adoption of APBI-brachy as an alternative to WBI in select patients.

S2-2
Luminal A Subtype Predicts Radiation Response in Patients with T1N0 Breast Cancer Enrolled in a Randomized Trial of Tamoxifen with or without Breast Radiation.

Fyles A, McCready D, Pintilie M, Shi W, Done S, Miller N, Olivotto I, Weir L, Liu F-F. Princess Margaret Hospital, Toronto, Canada; British Columbia Cancer Agency, Vancouver, Canada

Objectives
To determine the predictive effect of molecular subtyping using the six biomarker immunohistochemical panel in predicting ipsilateral breast relapse (IBR) in women age 50 and older with T1 and T2 node negative breast cancer in a randomized trial of tamoxifen (Tam) +/- whole breast radiation (WBRT).

Methods
Between December 1992 and June 2000, 769 women were randomized to WBRT and Tamoxifen (Tam, n=386) 20 mg daily for 5 years or Tam alone (n=383). Median age was 68 years, 639 (83%) had pT1 tumors. Intrinsic molecular subtype was determined using semi-quantitative analysis of ER, PR, Ki-67, HER2, EGFR and cytokeratin (CK) 5/6 on tissue microarrays constructed from tumor blocks from 172 of 345 available tumors. Patients were classified into the following categories: luminal A, luminal B, luminal-HER2, HER2 enriched, basal-like, or triple-negative phenotype-nonbasal. Median follow-up was 10 years.

Results
IBR at 10 years was 13.8% with Tam compared to 5.0% with Tam/ WBRT (p<0.0001). Tumour size (HR 1.54, p=0.001), ER positive (HR 0.35, p=0.006), age (HR 0.96, p=0.014), and treatment with Tam/WBRT (HR 0.28, p=0.0001) were significant factors for IBR. Luminal A tumors (ER or PR positive, HER2 negative, Ki-67<14%, n=95) had the lowest rate of IBR, 6.9% at 10 years with Tam alone and 4.5% with Tam/WBRT, p=0.4. In women aged 60 and over with Luminal A subtype IBR was 5.4% with Tam alone and 6% with Tam/ WBRT (n=74, p=0.8). Luminal B (ER or PR positive, HER2 negative, Ki67>14%, n=53) had an IBR of 23.8% with Tam alone and 0% with Tam/WBRT, p=0.012). Luminal HER2 (ER or PR positive, HER2 positive, n=10) and HER2-enriched (ER and PR negative, HER2 positive, n=14) demonstrated the highest risk of IBR.

Conclusions
Six marker IHC subtype appears to be predictive for radiation response in women over 50 with T1/2 node-negative breast cancer. Luminal A subtype demonstrated a low risk of breast relapse with Tam alone, particularly in women age 60 and older. These results require validation in additional specimens and clinical trials, but this subgroup represents a significant proportion of women (74/172 or 43%), who may be spared the inconvenience and side effects of breast radiation. In contrast, breast RT is beneficial in women with higher risk subtypes (Luminal B, HER2 enriched, basal). Limitations of this analysis include the relatively small numbers of patients and the low event rate in smaller subgroups.
S2-3
NSABP Protocol B-34: A Clinical Trial Comparing Adjuvant Clodronate vs. Placebo in Early Stage Breast Cancer Patients Receiving Systemic Chemotherapy and/or Tamoxifen or No Therapy – Final Analysis.

Paterson AHG, Anderson SJ, Lembersky BC, Feenbacher L, Falkson CI, King KM, Weir LM, Brufy AM, Dakhil S, Lad T, Baer-Diaz L, Gralow JR, Robidoux A, Perez EA, Zheng P, Geyer CE, Swain SM, Costantino JP, Mamounas EP, Wolmark N. National Surgical Adjuvant Breast and Bowel Project (NSABP); Tom Baker Cancer Centre; University of Pittsburgh Graduate School of Public Health; University of Pittsburgh Cancer Institute School of Medicine; Kaiser Permanente, Northern California; University of Alabama at Birmingham/ECOG; Cross Cancer Institute; British Columbia Cancer Agency: University of Pittsburg/Magee Women’s Hospital; Cancer Center of Kansas; Stroger Hospital Cook County MBCCOP; San Juan MBCCOP; University of Washington/SWOG; Centre Hospitalier de l’Université de Montréal; Mayo Clinic Jacksonville/NCCTG; Allegheny General Hospital; Washington Cancer Institute, Washington Hospital Center; Aultman Health Foundation

Bisphosphonates reduce the incidence of skeletal-related events (fractures, pain, hypercalcemia) in patients (pts) with bone metastases from breast cancer. By inhibiting osteoclast function and subsequent bone turnover they may inhibit the growth of bone (and other) metastases. Their role in preventing or delaying the development of bone (or other) metastases in pts with early breast cancer is uncertain.

Three previous trials of oral clodronate have given mixed results. The largest trial (placebo-controlled) and a smaller open-label trial suggested that oral clodronate benefitted pts with improved bone metastases-free survival (BMFS) and overall survival (OS), but a third open-label study showed no benefit with an apparent detrimental effect on survival. Studies of IV zoledronate in open-label trials in early breast cancer have also shown mixed results: the AZURE trial in pts with node positive breast cancer showed no benefit in disease-free survival (DFS) or OS with a possible effect in pts aged over 60. The ABCSG-12 trial in GnRH (plus tamoxifen or anastrozole) treated women with early breast cancer showed a small DFS benefit for those receiving IV zoledronate but no BMFS or OS benefit.

Methods: B-34 is a prospective, randomized, double-blind, phase III clinical trial in pts with stage 1, 2 or 3 breast cancer assessing oral clodronate 1600mg daily for 3 years compared to placebo given alone or in addition to adjuvant chemo- or hormone therapy. Stratification is by age (<50, >50), number of positive nodes (0, 1-3, >3) and ER/PR status. The primary end-point is DFS. Secondary endpoints are the incidence of skeletal metastases, OS, relapse-free survival, incidence of non-skeletal metastases, and incidence of skeletal morbid events.

Results: 3323 patients were accrued – 1662 in Group A and 1661 in Group B. As of March 31, 2011, 54 pts were declared ineligible: 23 in Group A and 31 in Group B. The average time on study is 101.3 months. Patient characteristics were evenly distributed throughout both groups. Approximately 75% of pts in each group had negative axillary lymph nodes and some 78% in each group were ER and/or PR positive. About 64% of pts were age 50 or over. Oral clodronate was generally tolerable, toxicities observed being mainly due to the concomitant systemic chemotherapy. One pt in Group A had a 1 mm area of osteonecrosis on the palatal tarsus. Compliance, as expected with trials of oral medications, has been a problem. 1910 pts completed 3 years of therapy. Event rates were slower than anticipated. This final analysis assesses a total of 598 events in the two arms.

Conclusions: This trial is the largest placebo-controlled study of an oral bisphosphonate in patients with early breast cancer and will provide further information on the role of bisphosphonates in breast cancer management. Supported by NSABP by NCI U10CA12027, -37377, 69651, 69974; and Bayer Schering Pharma Oy; for ECOG by U10CA021115; for NCCTG by U10CA25224; for SWOG by U10CA38926.

S2-4
GAIN (German Adjuvant Intergroup Node Positive) Study: A Phase-III Multicenter Trial To Compare Dose Dense, Dose Intense ETC (iddETC) vs. EC-TX and Ibandronate vs. Observation in Patients with Node-Positive Primary Breast Cancer – 1st Interim EFFICACY Analysis.


Background: We previously showed that intense dose-dense (idd) epirubicin (E), paclitaxel (T), cyclophosphamide (C) results in a superior DFS and OS compared to conventionally dosed EC-T in pts with primary breast cancer (PBC) and ≥4 involved lymph nodes (LN) (Möbus et al JCO 2010). In the GAIN study, the intense dose-dense strategy has been further investigated as well as the adjuvant application of ibandronate (I). We here report on the planned interim efficacy analysis after 50% (N>401) of the required events have occurred.

Methods: A prospective, multi-center, controlled, non-blinded, randomized phase III trial investigating ETC (E: 150 mg/m², T: 225 mg/m², C: 2500–2000 mg/m², i.v. day 1; 15 cycles each: A1; or EC→TX (E: 112.5 mg/m² + C: 600 mg/m², i.v. day 1, q 4 weeks × 12 cycles; A2). Pts were further randomized in a 2:1 ratio to receive ibandronate: 50 mg/day p.o. for 2 years (B1) or observation (B2). Pts received a primary prophylaxis with either epeoitin (I) or darbinofos (D) and pegilaglumin (L). For recruitment of 1500 pts prophylactic ciprofloxacin was implemented and the dose of C was reduced to 2000 mg/m². Eligibility: Females ≥18 and <65 years, histologically confirmed LN positive uni- or bilateral PBC; adequate surgery, ≥1 pos.LN; ECOG ≤2; written informed consent.

Primary objective: compare DFS A1 vs. A2 and B1 vs. B2. Secondary objectives: OS, safety, incidence of secondary primaries, and EFS in subgroups of hormone sensitivity and number of pos. LN between arms; assessment of compliance; determine prognostic factors. 3000 pts with 801 events were needed to show an increase of 5-year DFS from 75% to 79% for pts receiving EC→TX and 728 events to show an increase of 5-year DFS from 75% to 79.5% for pts receiving l, assuming a drop-out rate of 5%, α=0.05 (two-sided) and 1-ß =80%.
An interim analysis for both primary objectives was planned after 50% of the expected events occurred. Safety results have been reported previously (Möbus et al. SABCS 2009).

**Results:** 3023 patients were randomized between 06/2004 and 08/2008. 1512 received ETC and 1511 EC→TX. 29pts never started therapy, 14 in ETC, 15 in EC→TX. Median follow-up is 38.7 months. Median age was 50 years; pN1 (37.7%), pN2 (35.4%); pN3 (26.7%); 77.4% had ductal invasive carcinoma, 46.6% were grade 3; 76.7% had hormone receptor-positive tumors, 22% were HER2-positive. 405 events have occurred by 12.05.2011. 380pts relapsed and 25pts died w/o relapse. The interim futility boundary for chemotherapy was not crossed. For the ibandronate question the futility boundary was reached. There was no difference in DFS and OS between the patients with and without ibandronate (DFS log-rank p=0.593; HR 1.059; 95%CI 0.861-1.301; OS log-rank p=0.801 HR 0.961; 95%CI 0.705-1.31).

**Conclusion:** The GAIN study demonstrated that adjuvant ibandronate does neither improve DFS nor OS in primary node positive breast cancer after treatment with dose-intensified chemotherapy.

**S2-5**

**An Anti-HER3 Antibody That Stabilizes the Inactive Conformation Inhibits Both HER2 and Ligand Driven Tumor Growth.**

Garner AP, Bialucha CU, Chen D, Elif W, Kunz C, Li S, Martic J, Saxena P, Sineschekova O, Sprague E, Ettenberg S. Novartis Institutes for Biomedical Research, Cambridge, MA; Morphosys AG, Munich, Germany; Sanofi-Aventis, Cambridge, MA

Background: HER3 (ErbB3) is a member of the ErbB family of receptor tyrosine kinases (RTK). In normal physiology, ligands (e.g. neuregulin) activate HER3 by promoting dimerization with other RTK's such as HER2 (ErbB2). Inappropriate HER2/HER3 dimerization as a result of HER2 over-expression in cancer results in HER3 mediated activation of the oncogenic PI3K pathway. The HER2-targeted antibody trastuzumab inefficiently inhibits HER2 mediated HER3 activation allowing persistent HER3 signaling that is speculated to limit clinical responses. Consequently, combination of a HER3-targeted agent with trastuzumab may be of clinical benefit. Furthermore, ectopic HER3 activation has recently been implicated in the relief of a feedback loop induced by PI3K inhibitors that may perhaps limit their efficacy in HER2 driven tumors. Since HER3 activation in HER2 driven cancers occurs in a ligand-independent manner, antibodies that primarily inhibit ligand-induced HER3 activation are largely inactive in HER2 driven xenograft models.

Results: H3F15, a high affinity (26pM) HER3-targeted fully human IgG1 antibody was selected from the Human Combinatorial Antibody Library (HuCAL) using phage display technology. In a broad range of HER2 amplified breast and gastric cell lines, H3F15 displayed potent inhibition (IC50 <1nM) of HER3/ AKT phosphorylation and proliferation. Interestingly, H3F15 also effectively inhibited neuregulin stimulated HER3 phosphorylation and downstream signaling in MCF7 cells indicating that H3F15 inhibits multiple modes of HER3 activation. Determining the H3F15/HER3 crystal structure revealed that H3F15 binds a novel conformational epitope that traps HER3 in the inactive conformation thus preventing its activation by either HER2 or neuregulin. In vivo testing of H3F15 using xenograft tumor models driven by either HER2 (BT474) or HER3 ligands (BxPC3) confirmed that single agent H3F15 (20mg/kg) significantly inhibited tumor growth (83% and 77% inhibition respectively). Furthermore, combinations of H3F15 with either trastuzumab or PI3K-targeted agents were synergistic in a panel of HER2 driven cell lines whilst the in vivo combination of H3F15 (20mg/kg) with trastuzumab (1mg/kg) was sufficient to induce tumor regression.

Discussion: H3F15 is a HER3-targeted antagonist IgG1 that stabilizes the inactive form of HER3. This novel mechanism of action enables H3F15 to uniquely inhibit both ligand-induced and HER2-mediated activation of HER3. Thus H3F15 is the first HER3 antibody to display single-agent efficacy in both HER2 and ligand driven xenograft models whilst also inducing tumor regressions in combination with trastuzumab. Based on preclinical data, combining H3F15 with either trastuzumab or PI3K-targeted agents fully inhibits the HER2/HER3 signaling pathway, which may lead to greater and more sustained clinical efficacy in HER2 driven cancers.

**S2-6**

**ErbB3 Expression Is Required for Maintenance of Normal and Transformed Luminal Breast Epithelial Cells.**

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The ErbB receptor tyrosine kinase family regulates breast development and cancer. ErbB2/HER2 expression correlates with HER2-enriched breast cancers, while epidermal growth factor receptor (EGFR) expression often correlates with triple negative breast cancers. Less is known regarding ErbB3, which harbors only weak kinase activity, but strongly activates phosphatidylinositol-3 kinase (PI3K)/Akt signaling upon heterodimerization with EGFR or ErbB2. We report herein that ERBB3 mRNA expression strongly correlates with Luminal A/B breast cancers, which express lower levels of EGFR and ErbB2. Mammary-specific loss of ErbB3 in mice reduced Akt phosphorylation and caused cell death in the luminal epithelium. A decreased luminal population in ErbB3-deficient epithelium correlated with expansion of the mammary stem/progenitor fraction. In normal breast samples, ERBB3 mRNA expression was highest in mature/progenitor luminal populations, and lowest in the stem/basal population. Loss of ErbB3 shifted gene expression in mammary epithelial cells to resemble a mammary stem cell signature. The genes most greatly impacted by ErbB3 loss produced a signature that correlated with decreased overall survival in tamoxifen-treated patients. Knock-down of ErbB3 in human luminal breast cancer cells caused decreased cell growth under conditions of estrogen deprivation, decreased expression of the gene encoding aromatase, and impaired expression of estrogen receptor-induced genes, including those that encode progesterone receptor and TFF1. Treatment of MCF7 luminal breast cancer xenografts with AMG-888, a humanized monoclonal antibody directed against ErbB3, decreased tumor growth. Combination of AMG-888 with the estrogen receptor inhibitor fulvestrant caused MCF7 tumor regression. Taken together, these results suggest that ErbB3 is required to sustain normal and transformed luminal epithelial cells of the breast, and suggest that targeting ErbB3 may improve the clinical outcome of breast cancers treated with endocrine therapy.
S2-7
Mechanisms of Action and Biological Significance of HER2 Mutations in HER2-Overexpressing Breast Cancer.

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Background: Trastuzumab is the most successful HER2-directed therapy in patients with early-stage and advanced HER2 positive breast cancer. Although trastuzumab improves survival in the adjuvant setting, 15-20% of the patients develop metastasis. Lapatinib is currently the only tyrosine kinase inhibitor approved for the treatment of patients with metastatic breast cancer after trastuzumab progression. However, progression eventually occurs and the disease remains incurable for the majority of patients. Recently, somatic mutations in the HER2 kinase domain have been reported in lung adenocarcinomas which result in constitutive phosphorylation of HER2, EGFR and cellular substrates. Activation of these receptors and pathways in lung cancer was associated with tumor progression and trastuzumab resistance, but tumors remained sensitive to a tyrosine kinase inhibitor. Because there are many different types of breast cancer that respond differently to treatments, more approaches are needed to predict which patients will most likely respond to a given therapy.

Material and Methods: Using gene sequencing on 78 HER2 positive breast tumors, our laboratory identified 4 novel missense variants in the kinase domain of HER2. None of the patients whose tumors carried one of these mutations achieved objective response to trastuzumab. Mutants were created using directed mutagenesis, inserted in a lentiviral expression plasmid and stably expressed in different breast cancer or non-tumorigenic cell lines. An ATP-based assay was used for cell survival studies. Migration studies were performed using Boyden-chambers coated with Matrigel. Anchorage-independent colony formation was assessed in soft-agar. Signaling pathways and phosphorylation status were analyzed by Western blot. Localization results were obtained by immunofluorescence and confocal microscopy followed by deconvolution analysis.

Results: Two of the mutants were dramatically under-phosphorylated and presented an altered cellular localization revealed by immunofluorescence studies in both cell lines and patient surgical samples. Also, cells expressing HER2 mutants showed an increased ability to invade Matrigel and migrate and to form colonies in soft-agar, suggesting the induction of a more aggressive behavior. In particular, one of the mutations was strongly associated with resistance to lapatinib treatment in cell survival and soft-agar assays, and higher doses of lapatinib were necessary to inhibit the ERK and AKT pathways. Computational analysis revealed that the mechanism of lapatinib resistance could be explained by a sterical obstruction of the ATP-binding pocket of the protein kinase domain that would impede the binding of lapatinib.

Conclusion: HER2 mutations confer a more aggressive phenotype. A specific mutation directly interacts with Lapatinib binding to HER2 and predicts resistance to Lapatinib in HER2-overexpressing breast cancer cells. Further characterization of novel HER2 mutations may have a direct implication in the development of novel markers for early diagnostics, patient selection and characterization of more appropriate and personalized treatment.

S3-1
Update of International Breast Cancer Study Group Trial 23-01 To Compare Axillary Dissection Versus No Axillary Dissection in Patients with Clinically Node Negative Breast Cancer and Micrometastases in the Sentinel Node.


Introduction and Study Design
For patients (pts) with a metastatic sentinel node (SN), axillary dissection is standard treatment to achieve optimal locoregional control. However, for many pts the SN is the only positive node and for pts with minimal SN involvement, axillary dissection (AD) may be overtreatment. IBCSG Trial 23-01 was designed to determine whether AD is necessary in pts with minimal SN involvement (defined as one or more micrometastatic (<2 mm) SNs) and tumor ≤ 5 cm. Consenting eligible pts were first registered; those with the requisite SN involvement were randomized to AD (group A) vs. no further axillary surgery (group B). The primary endpoint was disease-free survival (DFS). Secondary endpoints included overall survival (OS) and systemic disease-free survival (SDFS). The trial started in April 2001 and closed in February 2010. The accrual target was 1,960 pts to provide 90% power to detect non-equivalence if 5-year DFS was 64% for group B and 70% in group A. At closure 6,681 pts had been registered, with 934 randomized from 27 centers. The primary reasons for early closure were that projected time to complete accrual was too long, and the aggregate event rate at 30 months median follow-up was much lower than anticipated.

Baseline Characteristics and Treatment
Mean patient age at entry was 54 years (range 26-81). More postmenopausal (56%) than premenopausal pts (44%) were randomized. Sixty-seven percent of pts had tumor <2 cm, while 7% had tumor ≥ 3 cm; 26% had grade 3 disease. Tumors were estrogen-receptor positive in 89% of pts, and progesterone-receptor positive in 75%. In the involved sentinel node(s), 67% of pts had ≤ 1.0 mm micrometastasis, 29% had 1.1-2.0 mm micrometastasis, 2% had metastasis >2.0 mm, and 2% were unknown. Most (96%) pts underwent lymphoscintigraphy, and 1 or 2 sentinel nodes were found in about 85%. A previous excision biopsy was performed in 16%. Conservative surgery was definitive treatment in 75%; the others received mastectomy. Adjuvant radiotherapy was performed in 89% of group A and 92% of group B.

Outcomes
On 25 May 2011, median follow was 49 months. There were 88 DFS events. Sites of first DFS event were breast cancer-related in 66 pts [local (8), contralateral breast (10), regional (6), and distant (42)], and non-breast cancer-related in 22 [second malignancies (17) and deaths without prior cancer event (5)]. Four-year DFS (+ standard error) was 91% (+1.4%). Four-year competing risk cumulative incidences were 7.3% (+1.0%) for breast cancer events and 2.0% (+0.5%) for non-breast cancer events. With 101 DFS events, the trial is estimated to have 90% power to detect non-equivalence if 5-year DFS is 87% for group B compared with 92% for group A.

Conclusion
In this trial, restricted to clinically N0 with microscopic SN involvement, breast cancer recurrence and relapse rates are very low at a median follow-up of 4 years. The first comparison of outcomes between the two arms will be presented after a median follow-up of 5 years, when number of DFS events is anticipated to exceed 100.
S3-2
Neoadjuvant Chemotherapy Adapted by Interim Response Improves Overall Survival of Primary Breast Cancer Patients – Results of the GeparTrio Trial.


Background:
The GeparTrio phase III trial investigated the concept of interim response-adapted neoadjuvant chemotherapy. Patients with an early response after 2 cycles chemotherapy were considered highly chemo-sensitive and randomized to additional 2 chemotherapy cycles compared to standard treatment. Patients with no early response were considered less chemo-sensitive and randomized to continue with a non-cross-resistant chemotherapy or with standard chemotherapy. Pathological complete response (pCR) rates were different between responders and non-responders but not between the randomized arms (von Minckwitz G, et al JNCI 2008+2008; Huober et al. BCRT 2010).

We report here on the results of the secondary endpoints: disease-free (DFS) and overall survival (OS).

Patients and Methods:
2072 patients with operable or locally advanced breast cancer were treated with 2 cycles TAC (docetaxel, doxorubicin cyclophosphamide) before interim response assessment. Responders were randomized to additional TACx4 (N=704) or TACx6 (N=686) and non-responders to TACx4 (N=321) or NXX4 (vinorelbine, capecitabine) (N=301). None of the HER2+ patients received Trastuzumab. Endocrine treatment was given postoperatively to ER+ and/or PgR+ patients. We observed 480 recurrences and 302 deaths during median 62 months of follow up.

Results:
Patients receiving the experimental treatments (TACx8 or TACx2-NXX4) showed a longer DFS (HR 0.71; 95%CI 0.60-0.86, p<0.001) and longer OS (HR 0.79; 95%CI 0.63-0.99, p=0.048) compared to patients receiving standard TACx6 treatment. Treatment effects on DFS were restricted to patients with luminal A (p=0.003), luminal B (HER2-) (p=0.006) and luminal B(HER2+) (p=0.04) tumors. Experimental treatments did not improve outcome in HER2+(non-luminal) (p=1.0) and triple-negative (p=0.5) tumors. Responders showed a significant longer DFS (HR 0.79; 95%CI 0.62-0.97, p=0.026) and a trend towards a longer OS (HR 0.76; 95%CI 0.57-1.01, p=0.061) if they were treated with TACx8 compared to TACx6. Non-responders showed a longer DFS (HR 0.6; 95%CI 0.43-0.82, p=0.001) but not OS (HR 0.85; 95%CI 0.57-1.27, p=0.4) when treated with TACx2-NXX4 compared to TACx6. Results according to phenotypes of responders and non-responders were comparable to the overall comparison. In general, patients with a pCR showed a better DFS if they had triple-negative (p<0.0001), HER2+(non-luminal) (p<0.0001) or luminal B(HER2-) (p=0.004), but not if they had luminal A (p=0.66) or luminal B (HER2+) (0.67) disease.

Conclusion:
Adapting neoadjuvant chemotherapy according to interim response leads to better DFS and OS and represents therefore a unique advantage over adjuvant treatment. The investigated strategies to improve standard chemotherapy were most effective in the luminal A and B phenotypes. These phenotypes are usually considered less chemo-sensitive and pCR is not a prognostic factor. This might explain why the observed survival advantages could not be predicted by pCR.

S3-3
Association of PTEN Loss and PIK3CA Mutations on Outcome in HER2+ Metastatic Breast Cancer Patients Treated with First-Line Lapatinib Plus Paclitaxel or Paclitaxel Alone.

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Background
Identifying molecular determinants underpinning response and resistance to anti-cancer therapies is essential to selecting optimal treatment for patients (pts). The PI3K pathway is frequently deregulated in cancer. In preclinical models deregulated PI3K has been shown to enhance the survival of breast cancer cells and confer resistance to chemotherapy and HER2-directed agents. This exploratory study evaluated the impact PIK3CA mutations and PTEN loss had on the efficacy of paclitaxel alone (P) and in combination with lapatinib (L+P) in HER2-positive metastatic breast cancer (MBC) pts enrolled in study EGF104535, results of which demonstrated that pts treated with the combination of L+P derived an absolute improvement in median overall survival (OS) of 7.3 months (median OS=27.8 months in L+P compared with 20.5 months in P).

Method
Baseline tissue from primary breast tumor or metastatic site was obtained in the form of formalin-fixed, paraffin-embedded material. Of the total study population (n=444), 389 pts had tissue available for biomarker analyses, 277 of whom provided written informed consent for optional tumor genetics (i.e., PIK3CA analysis). PTEN was evaluated on whole sections by immunohistochemistry (rabbit monoclonal antibody); pathology review and image analysis were performed, producing an ordinal score and optical density (OD). Qiagen/DxS assay was used in assessing PIK3CA mutation status. Logistic regression and Cox-proportional hazard models were used in analyses of biomarkers with efficacy endpoints.

Results
Evaluable results for PTEN and PIK3CA were available for 91% (354/389) and 62% (171/274) of pts, respectively. In the overall population, 24% (65/274) had tumors harboring PIK3CA mutations (ES42K, ES45K/D, H1047R); 13% (49/389) had absence of PTEN expression. Survival outcome appeared to be independent of PTEN expression (P>0.2). PIK3CA mutations were significantly associated with worse OS (HR=1.88; 95% CI=1.26, 2.80; P=0.002). DFS was significantly improved in pts treated with L+P compared with P in both PTEN strata (loss/expression; P<0.001). In pts stratified by PIK3CA status, treatment with L+P reduced the risk of progression compared with P in the wildtype strata (N=106; HR=0.43; 95% CI=0.28, 0.65; P<0.0001); in the mutation strata, PFS was not significantly reduced with the addition of L (N=65; HR=0.70; 95% CI=0.42, 1.17; P=0.179).
Conclusions
In this exploratory study, PTEN was not prognostic whereas PIK3CA mutations appear to be an adverse prognostic feature in HER2+/MBC. PTEN expression may not be predictive as both strata derived significant benefit with L+P treatment. PFS benefit from L+P was significant in pts with PIK3CA wildtype tumors whereas the PFS benefit was not significant in the PIK3CA mutation strata. Additional analyses are ongoing and will be presented.

S3-4
ER Downregulation with Fulvestrant in Combination with pan-PI3K Inhibitor BKM120 Synergizes Against ER+/PI3K-Mutant Breast Cancer Xenografts In Vivo.
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ER+ breast cancers typically respond to aromatase inhibitors (AIs), but a significant fraction exhibit de novo or acquired resistance. To discover mechanisms of resistance to AIs, we maintained four ER+, hormone-dependent human breast cancer cell lines in hormone-depleted conditions for several months until estrogen-independent populations emerged (termed long-term estrogen-deprived, LTED). A siRNA kinome screen identified 42 kinases that suppressed growth and/or proliferation in LTED cell lines, and ER downregulation with fulvestrant did not inhibit growth in these 42 kinases. Further, treatment with the PI3K/mTOR dual inhibitor BEZ235 or the pan-PI3K inhibitor BKM120 suppressed LTED cell growth, and prevented the emergence of hormone-independent cells. Activating mutations in PIK3CA, the gene encoding the p110α catalytic subunit of PI3K, are present in about 35% of ER+ breast cancers. We investigated whether PIK3CA mutations modify the tumor response to estrogen deprivation with letrozole in a pre-surgical clinical trial (NCT00651976). Twenty-one patients with stage I-II operable ER+/HER2- breast cancer were treated with letrozole for 10-21 days up to 24 h prior to surgery. Core tumor biopsies were obtained before treatment and at the time of surgical resection. Ki67 immunohistochemistry (IHC) was scored in pre- and post-letrozole specimens. The change in natural log of the Ki67 score between pre- and post-treatment samples was stratified by PIK3CA mutant vs. wildtype (WT) status. PIK3CA-mutant tumors exhibited a statistically lower reduction in Ki67 score compared to tumors with WT PIK3CA (p<0.05). These findings suggest that mutational activation of PI3K attenuates the response to estrogen deprivation.

In some LTED cell lines, downregulation of ER with fulvestrant or siRNA inhibited estrogen-independent growth, suggesting that the unliganded ER can maintain proliferation of cells adapted to estrogen deprivation. Prior work suggested that PI3K-ER crosstalk promotes resistance to estrogen deprivation. However, inhibition of PI3K/AKT/TORC1 signaling did not alter ER5167 phosphorylation in LTED cells, and ER downregulation with fulvestrant did not inhibit PI3K/AKT, suggesting that PI3K and ER modulate non-overlapping pathways in cells adapted to estrogen deprivation. Treatment with BKM120 synergized with fulvestrant to inhibit the growth of ER+/PI3K-mutant MCF-7 xenografts, while the combination induced near-complete tumor regression. IHC analysis showed increased cleaved caspase-3+ cells in BKM120-treated tumors and decreased Ki67+ cells in fulvestrant-treated tumors, implying that the combination induced tumor regression by both inhibition of tumor cell proliferation and induction of apoptosis. These data suggest that upon progression on an aromatase inhibitor, patients with ER+/PI3K-mutant breast cancer would benefit from a combination of an ER downregulator and a PI3K pathway inhibitor.

S3-5
Next Generation Sequencing Reveals Co-Activating Events in the MAPK and PI3K/AKT Pathways in Metastatic Triple Negative Breast Cancers.
INTRODUCTION: The clinical application of next generation sequencing to comprehensively characterize groups of driving mutations in individual metastatic triple negative breast cancer (mTNBC) genomes has the potential to reveal therapeutically relevant pathway dependencies. Towards this end, we harvested tissue from 14 patients with mTNBC and are conducting deep whole genome and transcriptome sequencing for each case to identify mutations that can guide therapeutic targeting within available phase I/II clinical trials.
METHODS: Metastatic tumor tissue was harvested from 14 mTNBC patients, and 7 samples have undergone total genome and transcriptome sequencing with the others currently underway. We are utilizing the Life Technologies SOLID® system to sequence germline and tumor DNA to sufficient depth to identify somatic genome alterations including point mutations, indels, and structural events including translocations. Furthermore, RNA-seq is being performed on these tumors, along with a series of age- and ethnicity-matched normal breast controls to perform deep differential expression analysis, isoform expression analysis, and fusion transcript detection. Our team of genome scientists and clinical oncologists are evaluating the sequencing findings and are prioritizing the investigational therapeutic options for each patient.
RESULTS: Our whole genome and transcriptome sequencing study has revealed numerous known and novel mutations in mTNBC. However, all patients’ cancers analyzed to date had alterations that would activate the MAPK pathway, but through various mechanisms in different patients. These include BRAF amplification and overexpression, NF1 homozygous deletion, and consistent IQGAP3 overexpression. Furthermore, all patients’ cancers also harbor mutations that would activate the PI3K/AKT pathway including PTEN homozygous deletion or down-regulation, consistent INPP4B amplification/overexpression. However, we and others show ERBB4 down-regulation in breast tumors, we are the first to report unique somatic genomic events that significantly alter the ERBB4 locus leading to its loss in the majority (5/7) of our patients’ tumors. Importantly, we are beginning to use these insights to prioritize therapeutic targeting and have observed that one chemotherapy-refractory mTNBC patient, with a high-level BRAF amplification/overexpression along with down-regulation of PTEN and INPP4B, had a major response to combined mek plus akt inhibitors on a phase I study.
CONCLUSIONS: Comprehensive genomic and transcriptomic interrogation of mTNBCs has revealed events supporting co-activation of the MAPK and PI3K/AKT pathways in all the tumors.
albeit by different mutational mechanisms and supports potential effectiveness of combination therapy in the treatment of mTNBC. We plan to treat these patients with combined mek plus akt inhibitors on a new phase I study beginning in August 2011 to determine the effectiveness of co-inhibition of these pathways based on this frequent genomic context.

**S3-6**

**Neoadjuvant Chemotherapy of Paclitaxel with or without Rad001: Results of the Non-Responder Part of the GEPAQUINTO Study (GBG 44).**


**Background:**

The oral signal transduction inhibitor everolimus (RAD001 = R), binds selectively to mTOR (mammalian target of rapamycin), an intracellular protein kinase implicated in the control of cellular proliferation of activated T-lymphocytes and neoplastic cells. Phase II data suggested that R can enhance the clinical efficacy of endocrine treatment in the metastatic and neoadjuvant setting. The GeparQuinto phase III study had 3 settings (HER2-positive: trastuzumab vs lapatinib; HER2-negative: +/- bevacizumab (Bev); HER2-negative non-responder: +/- R). Primary aim of the last setting was to improve pathological complete response (pCR) for patients with HER2-negative breast cancer not responding to 4x epirubicin/cyclophosphamide (EC) +/- Bev by adding R to weekly paclitaxel as neoadjuvant chemotherapy.

**Patients and Methods:**

Patients with untreated HER2-negative breast cancer were eligible if their tumors were stage cT3/4a-d; or estrogen (ER) and progesterone (PgR) receptor-negative; or ER/PgR-positive tumors with clinically N+ (for cT2) or pN+ (for cT1). Only patients without response (<50% tumor reduction) to 4x EC +/- Bev were eligible and were randomized to receive further paclitaxel (Pac: 80 mg/m² q1w x12) chemotherapy with or without R. Treatment with R started 21 to 35 days after the last application of EC with a dose escalation from 2.5 mg every 2nd day to 5 mg every day over 14 days and maintained at 5 mg/day for additional 10 weeks. Dose of R could be decreased days after the last application of EC with a dose escalation from 2.5 mg every 2nd day to 5 mg every day over 14 days and maintained at 5 mg/day for additional 10 weeks. Dose of R could be decreased to 2.5 mg/day in case of toxicity. Treatment with Pac started within 7-14 days after the start of R. pCR was defined as no invasive and no non-invasive tumor residuals in breast and nodes. We assumed a pCR rate of 5% for Pac based on the GeparDuo study and expected a pCR of 12.1% for Pac+R (odds ratio 2.62). A two-sided Pearson’s C with α=0.05 and β=0.20 calculated a sample size of 566 P. One interim futility analysis after 1/3 of patients completed therapy was planned. Randomization was stratified by participating center, ER/PgR status, extend of disease (T4 or N3 vs. T1-3 and N0-2) and pre-treatment with Bev or not.

**Results:**

Between 11/07 and 15/06/11 402 P were randomized to Pac (N=201) and Pac+R (N=201). Median age was 51.0 and 50.0 [-R / +R] years. Median clinical tumor size was 40 / 40 mm; 62% / 55% had cT2, 18% / 20% cT3, and 16.7% / 16.7% cT4a-d tumors; 88% / 89% had non-lobular; 35% / 33% grade 3; 55% / 57% node-positive; and 29% / 27% ER and PgR-negative (triple-negative) disease.

The futility interim analysis was performed in 02/10, futility boundary was not reached and the trial was continued. After the other 2 settings completed accrual in 06/10, recruitment to the 3rd setting dropped such that it appeared not possible to recruit the full number of patients. The trial will therefore close recruitment on June, 30th 2011 with an estimated statistical power of 65%. Results on histological response and surgical outcome will be reported.

**Conclusion:**

This will be the first report on efficacy data of neoadjuvant R + Pac for patients with early breast cancer. The results of the GeparQuinto study will have to be set into context with the results from the Bolero studies in metastatic disease.

**S3-7**

**Everolimus for Postmenopausal Women with Advanced Breast Cancer: Updated Results of the BOLERO-2 Phase III Trial.**

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**Background:**

The mTOR pathway is constitutively activated in hormone-resistant advanced breast cancer (ABC). In phase II trials, everolimus (EVE) showed promising efficacy both as monotherapy and in combination with endocrine therapy in patients with estrogen receptor–positive (ER+) ABC. This double-blind, placebo-controlled, phase III study evaluated EVE plus exemestane (EXE) in patients with ER+ ABC refractory to letrozole or anastrozole.

**Patients and Methods:**

Eligible patients were randomized (2:1) to EXE (25 mg/day) with EVE (10 mg/day) or with matching placebo. The primary endpoint was progression-free survival (PFS). Secondary endpoints included overall survival, response rate, quality of life (QoL), and safety.

**Results:**

724 patients were randomized (485: EVE+EXE; 239: EXE). Baseline characteristics were well balanced; median age was 62 years, 56% had visceral involvement, and 84% had documented benefit from previous endocrine therapy, which included letrozole or anastrozole (100%), tamoxifen (48%), fulvestrant (16%), and chemotherapy for advanced disease (25%). This analysis is based on 457 events and median follow-up of 12.5 months. PFS by investigator assessment showed a hazard ratio (HR) of 0.44 (95% CI: 0.36-0.53) and a median duration of 7.4 (EVE+EXE) vs 3.2 months (EXE) (P<1 × 10^-9) and 12-month estimate of 31% vs 10%. PFS by central assessment showed an HR of 0.36 (95% CI: 0.28-0.45) and a median duration of 7.4 (EVE+EXE) vs 3.2 months (EXE) (P<1 × 10^-9) and 12-month estimate of 48% vs 18%. Response rates and clinical benefit rate were also higher for EVE+EXE (12.0% vs 1.3% and 50.5% vs 25.5%) vs EXE. A total of 138 patients died; 17.3% in the EVE+EXE arm and 22.6% in the EXE arm.

The most common grade 3/4 adverse events were stomatitis (8% vs 1%), anemia (7% vs 1%), hyperglycemia (5% vs 1%), dyspnea (4% vs 1%), and fatigue (4% vs 1%) for the EVE+EXE and EXE groups, respectively. Grade 3 pneumonitis was observed in patients receiving EVE (3% vs 0%). No difference in time to deterioration of QoL was
observed. EVE increased EXE steady-state C\text{max} and C\text{ss} levels by 45% and 64%, respectively, with no difference in estradiol levels. Serum markers of bone resorption and bone formation increased in the EXE arm and generally decreased in the EVE+EXE arm. **Conclusion:** The addition of EVE to EXE is associated with significant and sustained prolongation of PFS. Adverse events were higher in the combination arm but manageable by dose interruption and/or reduction and did not affect QoL. EVE in combination with an aromatase inhibitor is a promising therapeutic option for women with hormone receptor–positive advanced breast cancer.

**S4-1**

**Overdiagnosis in Breast Cancer Screening: Methodological Considerations of Current Estimates.**

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**Background**

A harm that has received considerable attention in breast cancer screening is overdiagnosis, i.e., the detection through screening of breast cancer that is non-progressive. Since there is no way to distinguish a truly non-progressive tumor from one that is progressive, overdiagnosis is a statistical phenomenon that is estimated as the difference between observed and expected incidence rates. However, there is no agreement on the true rate of overdiagnosis in breast cancer screening. Estimates vary widely, with the highest estimates reported in the scientific literature having received significant media attention under the premise that a significant fraction of newly diagnosed breast cancers are non-progressive and thus overtreated. We examined the range of overdiagnosis estimates in the context of factors that influence observed and expected breast cancer incidence rates over time.

**Methods**

Only studies that compared incidence rates of invasive breast cancer, or invasive breast cancer and ductal carcinoma in situ (DCIS), in a group invited or exposed to mammography compared with a group not invited or not exposed to mammography were included in our analysis. Studies were evaluated based on whether or not they adjusted for contemporaneous trends in the underlying incidence of disease, which may differ in different age groups, and age-specific effect on incidence rates due to lead time.

**Results**

We identified 17 studies (Table 1) that attempted to estimate overdiagnosis of breast cancer resulting from mammography screening. The main determinant of high estimated rates of overdiagnosis was a failure to adjust for lead time. The highest rate of overdiagnosis (33%) occurred in the one study that did not correct for either lead time or confounders. Studies that only partially corrected for lead time and confounding also had high estimates of overdiagnosis (mean = 30%), whereas studies that corrected for both lead time and confounders had the lowest estimate of overdiagnosis (mean = 5%).

**Conclusion**

The possibility that some breast cancers detected by mammography are indolent and not life threatening within the patient’s natural life is real. However, it is clear from our analysis that the wide range of estimates of overdiagnosis are due to whether or not studies have adjusted for factors in and outside of screening programs known to influence incidence rates over time. Our findings suggest that the true rate of overdiagnosis associated with breast cancer screening, to the extent that it exits at all, is small.

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**S4-2**

**The Risk of Contralateral Breast Cancer in BRCA1/2 Carriers Compared to Non-BRCA1/2 Carriers in an Unselected Cohort.**

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**Background:** Women who survived their first breast cancer have a higher risk to develop a new primary tumor in the contralateral breast than the risk of women in the general population to develop a first breast cancer. Especially women who carry a germline mutation in either the BRCA1 or the BRCA2 gene face a high lifetime risk of developing a synchronous or metachronous bilateral breast cancer. It is important to provide precise risk estimates of contralateral breast cancer and identify factors which predict the risk of CBC in this group of high-risk women. To answer these questions, we looked at the effect of BRCA1/2-carriership and its interaction with other factors on the risk to develop a CBC in an unselected cohort of breast cancer patients.

**Materials and methods:** We collected clinico-pathological, treatment and follow-up data for 4856 patients with unilateral, invasive breast cancer, diagnosed under the age of 50, between 1970 and 2003, in ten different hospitals throughout The Netherlands. Germline DNA was isolated from formalin-fixed paraffin-embedded tissue and patients were tested for the most prevalent pathogenic BRCA1 and BRCA2 mutations in The Netherlands. DNA and clinical data were coded before the analyses. All second primary breast tumors in the contralateral breast diagnosed more than 3 months after the diagnosis of the first breast cancer were considered as events. Preliminary results from life-table analysis and Cox Proportional Hazard models adjusted for age at diagnosis are shown here. Further statistical analyses will include competing risk analysis.

**Results:** In 4856 patients genotyped for BRCA1/2 mutations, 206 (4.2%) carriers were identified. During a median follow-up of 9.8 years (range 0-38), 9% of the patients developed a CBC, resulting in a cumulative 15-year risk for CBC of 10.4% (95% CI = 9.25-11.7) for non-carriers and 35.4% (95% CI = 25.9-46.9) for carriers of a BRCA1 or BRCA2 mutation (HR = 4.04 (95% CI = 2.88-5.68)). Patients carrying a BRCA1/2 mutation who were diagnosed under the age of 40 with their first breast cancer experienced a cumulative 15-year risk for CBC of 52.4% (95% CI = 36.4-70.3) versus 21.3% (95% CI = 12.0-36.0) in those over the age of 40 (HR = 0.30 (95% CI = 0.14-0.65)). Furthermore, BRCA1/2 mutation carriers with a triple negative first tumor had a cumulative risk for CBC of 43.6% (95% CI = 25.1-67.7), in contrast, BRCA1/2 mutation carriers with a non-triple negative first tumor had a cumulative risk for CBC of 13.4% (95% CI = 4.21-38.4) (HR = 0.24 (95% CI = 0.07-0.86)). Age at diagnosis and triple negative status were not found to be predictors of the risk of CBC in non-carriers (HR = 0.81 (95% CI = 0.53-1.24) and HR = 1.49 (95% CI = 0.91-2.41) respectively).

**Discussion:** In this study we identified subgroups of patients with a high risk to develop a CBC after their first breast cancer. Guidelines about treatment decisions and screening for follow-up should take into account these high risk subgroups to provide even better information and counseling for BRCA1/2 mutation carriers.

On behalf of more than 20 involved authors of the BOSOM study from 10 different hospitals and institutions throughout The Netherlands.
S4-3
Prospective Comparison of Risk Assessment Tools in Early Breast Cancer (Recurrence Score, uPA/PAI-1, Central Grade, and Luminal Subtypes): Final Correlation Analysis from the Phase III WSG-Plan B Trial.
Gluz O, Kreipe H, Degenhardt T, Kates R, Christgen M, Liedtke C, Shak S, Clements M, Markmann S, Ueber C, Augustin D, Thomssen C, Nitz U, Harbeck N. West German Study Group, Moenchengladbach, Germany; Medizinische Hochschule, Hannover, Germany; University of Muenster, Muenster, Germany; Genomic Health Inc, Redwood City; Klinikum Mutterhaus, Trier, Germany; Klinikum Suedstadt, Rostock, Germany; Gynecology Practice, Hildesheim, Germany; Klinikum Deggendorf, Deggendorf, Germany; University of Halle/ Saale, Halle/Saale, Germany; Bethesda Clinics, Moenchengladbach, Germany; University of Cologne, Cologne, Germany

Background: Both the Recurrence Score® (RS) multi-gene assay and invasion factors uPA/PAI-1 are recommended by guidelines (ASCO, AGO) for decision support regarding adjuvant chemotherapy (cht) in patients with early breast cancer (BC). In the EC-DOC trial, we previously suggested central grade 3 and high Ki-67 levels as independent predictive markers for enhanced benefit of taxane-based cht in intermediate risk BC. Here, we present the final WSG-Plan B trial correlation analysis of these risk assessment tools.

Methods: Plan B trial (evaluating anthracycline-free adjuvant cht, 6xTC, vs. 4xEC-4xDOC in HER2-negative BC; n=2,448 was randomized for chemotherapy). RS has been used as the selection criterion for cht vs. endocrine therapy alone in HR+ BC (if RS<11 in pN0 or pN1) since an amendment in August 2009. uPA/PAI-1 by ELISA is obtained as an optional risk factor. Evaluation of central grade and luminal B subtype (using 14% or 20% Ki-67 cut-offs) are performed by the independent trial pathologist in all HR+ tumors.

Results: From April 2009 to June 2011, 3037 patients have been recruited and 2290 randomized. The study will be completed by December 2011. Data on RS are available in 2361 patients with HR+ tumors: RS is distributed as follows: 0-11 (18%), 12-25 (61%), >25 (21%). In 257 patients with 0-3 involved LN, cht was omitted based on RS results (12.3% of patients after amendment).

The last interim analysis in February 2011, data on central grade are available in 1509 patients and Ki-67 in 592 patients. An only moderate positive correlation was observed between Ki-67 and RS (Spearman’s coefficient rs=0.336; p<0.001) as continuous variables and between RS and central grade (rs=0.498, p<0.001). High RS is predictive of high grade G3 (66% of RS>)(25% with high risk and 74% of RS=) high risk are G3), but 30-33% of tumors were assessed as G3 within S4-4
Clarioking the Risk of Breast Cancer in Women with Atypical Breast Lesions.
Coopey SB, Mazzola E, Buckley JM, Sharker J, Belli AK, Kim EMH, Polufriganoj F, Parmigiani G, Garber JE, Smith BL, Gadd MA, Specht MC, Guidi AJ, Roche CA, Hughes KS. Massachusetts General Hospital, Boston, MA; Dana-Farber Cancer Institute, Boston, MA; Newton-Wellesley Hospital, Newton, MA; Wayne State University, Detroit, MI

Background: Women diagnosed with atypical ductal hyperplasia (ADH), atypical lobular hyperplasia (ALH), lobular carcinoma in situ (LCIS), and borderline ADH/DCIS are at increased risk for breast cancer, but the precise degree of risk varies widely in the literature.

Information from prior studies is limited by grouping ADH and ALH together and by small cohort sizes.

Objectives: To identify women with a pathologic diagnosis of ADH, ALH, LCIS, and borderline ADH/DCIS using Natural Language Processing. To evaluate breast cancer risk based on atypia type.

Methods: Using Natural Language Processing, we reviewed all electronically available pathology reports from Massachusetts General Hospital, Brigham and Women’s Hospital, and Newton-Wellesley Hospital (members of Partners HealthCare System) from 1987-2010. We identified all women with a diagnosis of ADH, ALH, LCIS, and borderline ADH/DCIS with no prior or concurrent diagnosis of breast cancer. We determined the incidence of subsequent invasive and noninvasive breast cancer, the side of cancer diagnosis compared to original atypia side, and the time to cancer diagnosis for each atypia type.

Results: We reviewed 76,333 path reports in 42,950 unique individuals and identified 3049 women who were diagnosed with atypical breast lesions over this 14-year period; 1233 (40.4%) had ADH, 851 (27.9%) had ALH, 595 (19.5%) had LCIS, and 370 (12.1%) had borderline ADH/DCIS. The mean age for atypia diagnosis was 51 years (range: 18-93). At a mean follow-up of 66 months, cancer occurred in 7.0% of women with ADH, 11.3% of women with ALH, 11.1% of women with LCIS, and 8.4% of women with borderline ADH/DCIS. The median time to breast cancer diagnosis was 48 months with ADH, 50 months with ALH, 47 months with LCIS, and 60 months with borderline ADH/DCIS. Significantly more ipsilateral cancers developed than contralateral cancers for all types of atypia combined (p<0.027).

<table>
<thead>
<tr>
<th>Atypia Type</th>
<th>5-Year Cancer Risk</th>
<th>10-Year Cancer Risk</th>
<th>Number of Cancers</th>
<th>Percent of Ipsilateral Cancer</th>
<th>Percent of Contralateral Cancer</th>
<th>Percent of Bilateral Cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADH (n=1253)</td>
<td>4.7%</td>
<td>14.5%</td>
<td>From ADH (n=86)</td>
<td>65.9%</td>
<td>50.7%</td>
<td>31.2%</td>
</tr>
<tr>
<td>ALH (n=851)</td>
<td>9.5%</td>
<td>20.3%</td>
<td>From ALH (n=96)</td>
<td>55.2%</td>
<td>42.7%</td>
<td>1.0%</td>
</tr>
<tr>
<td>LCIS (n=595)</td>
<td>9.1%</td>
<td>16.4%</td>
<td>From LCIS (n=66)</td>
<td>56.1%</td>
<td>40.9%</td>
<td>0.0%</td>
</tr>
<tr>
<td>Borderline (n=370)</td>
<td>9.9%</td>
<td>14.2%</td>
<td>From Borderline (n=31)</td>
<td>54.3%</td>
<td>41.9%</td>
<td>3.2%</td>
</tr>
</tbody>
</table>

Table 1: Estimated 5 and 10-year breast cancer risks and laterality of cancer based on atypia type. The development of invasive versus noninvasive breast cancer was not significantly affected by atypia type. Subsequent cancers were DCIS in 121 patients (43.4%) and invasive in 158 patients (56.6%). Kaplan Meier curves for time to cancer diagnosis based on atypia type were created. The curves for ADH and borderline ADH/DCIS were similar and significantly different than the curves for ALH and LCIS (p<0.001). The estimated 5 and 10-year breast cancer risks for each atypia type are presented in Table 1.
Conclusion: A diagnosis of ADH, ALH, LCIS, or borderline ADH/DCIS increases a woman’s risk of invasive and noninvasive breast cancer in either breast. The breast cancer risk at 5 and 10 years is significantly higher in those with ALH or LCIS compared to those with ADH or borderline ADH/DCIS, but there is little difference in risk between ADH and borderline ADH/DCIS or between LCIS and ALH.

S4-5
Comparison of PAM50 Risk of Recurrence (ROR) Score with OncotypeDx and IHC4 for Predicting Residual Risk of RFS and Distant-(D)RFS after Endocrine Therapy: A TransATAC Study.

Methods: PAM50 analysis was conducted using NanoString technology on 125 ng RNA extracted from all 1026 samples from both N0 and N+ patients.

Background: OncotypeDx Recurrence Score (RS) is used to assess RR after endocrine therapy in primary ER+ breast cancer and is valid for both tamoxifen and anastrozole (Dowsett ref). IHC4 is a 4-panel set of IHC markers (ER, PgR, HER2, Ki67) that was shown to provide as much prognostic accuracy as RS in the translational arm of the ATAC trial (TransATAC) of anastrozole versus tamoxifen alone or combined and subsequently independently validated (Cuzick et al, JCO, 2011, in press). PAM50 is 50-gene test that has been optimised to separate intrinsic disease subtypes and is used to generate a ROR score. Good correlation has been shown between the ROR and RS but no large scale comparisons of their ability to predict clinical outcome have been conducted.

Methods: PAM50 analysis was conducted using NanoString technology on 125 ng RNA extracted from all 1026 samples from the TransATAC study that had at least 500ng RNA available. Nodal status was known on 986: N+, 271; N-neg 715. ROR scores were calculated and their relationship with RFS and DRFS assessed in both the N-neg and N+ categories. The prognostic value of ROR when added to standard clinical variables was assessed using the likelihood ratio chi-square. Finally, the relative accuracy of prognosis in this patient population using ROR, RS and IHC4 was evaluated by comparing the C-index for each test.

Results: Results are currently being derived and will be presented in full.

Discussion: This is the first large-scale clinical comparison of 2 prominent multi-gene predictors for use in FFPE material. The results will be instrumental in defining the preferred method for use in assessing residual risk of recurrence in patients treated with endocrine therapy.

S4-6
A Quantitative Multigene RT-PCR Assay for Predicting Recurrence Risk after Surgical Excision Alone without Irradiation for Ductal Carcinoma In Situ (DCIS): A Prospective Validation Study of the DCIS Score from ECOG E5194.

Methods: ECOG E5194 included 670 eligible patients with DCIS treated with surgical excision (≥ 3 mm negative margins) without irradiation, 228 of whom received tamoxifen. Patients had low or intermediate grade DCIS ≥ 2.5 cm, or high grade DCIS ≤ 1 cm. The Oncotype DX® assay was performed by quantitative RT-PCR using formalin fixed paraffin embedded tumor specimens from 327 patients (49% of the parent study). Recurrence Score® (RS) was calculated using the published algorithm. A new, prespecified DCIS Score™ was designed to predict recurrence using an optimized gene expression algorithm. The primary objective was to determine whether there was a significant association between the risk of an ipsilateral breast event (IBE) and the continuous DCIS Score in Cox models. 46 patients had an IBE (defined as ipsilateral local recurrence of DCIS [n=20] or invasive cancer [n=26]). Median follow-up was 8.8 years.

Results: The 10-year IBE rates were 15.4% for low/intermediate grade DCIS and 15.1% for high-grade DCIS (as determined by central pathology review), and for invasive IBE, 5.6% and 9.8%, respectively. Comparison between local and expert grading showed substantial disagreement. Continuous DCIS Score was significantly associated with IBE (HR 2.34 per 50 units; 95% CI 1.15, 4.59; p=0.02) when adjusted for tamoxifen use (prespecified primary analysis) and with invasive IBE (HR 3.73; CI 1.34, 9.82; p=0.01). DCIS Score was significantly associated with outcome when evaluated by the prespecified risk groups (see Table). Similar results were observed with and without adjustment for tamoxifen use or for negative margin width. Features associated with IBE in multivariate models included menopausal status (HR 0.49; 95% CI 0.27, 0.90; p=0.02), tumor size (HR 1.52 per 5 mm; 95% CI 1.11, 2.01; p=0.01) and continuous DCIS Score (HR 2.41; 95% CI 1.15, 4.89; p=0.02). The standard RS, which is calculated using thresholding of many genes unlike the DCIS Score, was not associated with IBE or invasive IBE (p > 0.6).

Conclusions: We have prospectively validated a multigene assay that quantifies recurrence risk and complements traditional clinical and pathologic factors in selected patients with DCIS treated with surgical excision without irradiation. The DCIS Score provides a new clinical tool for individualized selection of treatment for patients with DCIS.

10-Year Outcomes with the New DCIS Score

<table>
<thead>
<tr>
<th>DCIS Score Risk Group</th>
<th>10-Year Kaplan-Meier Rate (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Invasive Ipsilateral Breast Event</td>
</tr>
<tr>
<td></td>
<td>(Invasive or DCIS)</td>
</tr>
<tr>
<td>Low (&lt;55)</td>
<td>20.4% (13.8%, 30.2%)</td>
</tr>
<tr>
<td>Intermediate (30-55)</td>
<td>27.5% (15.2%, 45.9%)</td>
</tr>
<tr>
<td>High (&gt;55)</td>
<td>45.0% (29.9%, 68.5%)</td>
</tr>
</tbody>
</table>

Log rank p-value | 0.01
S4-7
Results of a Randomized, Double-Blind, Multicenter, Placebo-Controlled Study of Adjuvant Lapatinib in Women with Early-Stage ErbB2-Overexpressing Breast Cancer.
Goss P, Smith I, O'Shaughnessy J, Ejlertsen B, Kaufmann M, Boyle F, Buzdar A, Fumoleau P, Gradishar W, Martin M, Moy B, Piccart-Gebhart M, Pritchard K, Aktan G, Rappold E, Williams L, Finkelstein D. Massachusetts Gen Hosp, Boston, MA; Royal Marsden Hosp, London, United Kingdom; Baylor Sammons CancerCtr, Dallas, TX; Rigshospitalet, Copenhagen, Denmark; JW Goethe-Universität, Frankfurt, Germany; Royal North Shore Hosp, Sydney, Australia; UT MD Anderson Cancer Ctr, Houston, TX; Centre GF Leclerc, Dijon, France; Northwestern Univ, Chicago, IL; Hosp Universitario San Carlos, Madrid, Spain; Jules Bordet Inst, Brussels, Belgium; Toronto-Sunnybrook Regional Cancer Ctr, Toronto, ON; GlaxoSmithKline, Collegeville, PA and Uxbridge, United Kingdom

Late-breaking – abstract will be available at the meeting.

S4-8
First Results of AVEREL, a Randomized Phase III Trial To Evaluate Bevacizumab (BEV) in Combination with Trastuzumab (H) + Docetaxel (DOC) as First-Line Therapy for HER2-Positive Locally Recurrent/Metastatic Breast Cancer (LR/mBC).
Gianni L, Romieu G, Lichinitser M, Serrano S, Manssuti M, Pivot X, Smirnova I, Moliterni A, Andre F, Chan A, Lipatov O, Chan S, Wardley A, Greil R, Provencher L, Moore N, Prot S, Semiglazov V. Ospedale San Raffaele, Milan, Italy; Centre Régionale de lutte Contre le Cancer, Val d'Aurelle, Montpellier, France; N. N. Blokhin Russian Oncology Research Center, Moscow, Russian Federation; Fundação Pio XII- Hospital do Cancer de Barretos, Barretos, Brazil; University Hospital of Udine, Udine, Italy; University Hospital, Montpellier, France; Medical Radiological Science Center, Obninsk, Russian Federation; Fondazione IRCCS, Istituto Nazionale Tumori, Milan, Italy; Gustave Roussy Institute, Villejuif, France; Mount Hospital, Perth, Australia; Republic Clinical Oncology Dispansary, Ufa, Russian Federation; Nottingham University Hospitals NHS Trust, Nottingham, United Kingdom; The Christie, Manchester, United Kingdom; III. Medizinische Universitätsklinik Salzburg, Salzburg, Austria; CHA-Hôpital du Saint-Sacrement, QC, Canada; F. Hoffmann-La Roche Ltd, Basel, Switzerland; N. N. Petrov Research Institute of Oncology, St Petersburg, Russian Federation

Background: H + taxane is an established and effective first-line treatment for HER2-positive LR/mBC. Preclinical data provide the rationale for combining BEV and H in HER2-positive LR/mBC but clinical data in this setting are limited to single-arm phase II studies. AVEREL is the first randomized trial of BEV in HER2-positive LR/mBC.

Methods: Eligible patients had measurable or evaluable HER2-positive LR/mBC (centrally confirmed IHC 3+ or FISH/CISH +), ECOG performance status 0/1, and had received no prior chemotherapy for advanced disease. Prior adjuvant H/taxanes were permitted unless disease had recurred ≤6/>12 months after the last dose, respectively. Patients with CNS metastases were excluded. Patients were stratified according to adjuvant H, prior (neo)adjuvant taxane (further stratified by time to relapse since last dose of [neo] adjuvant chemotherapy/no chemotherapy), hormone receptor status, and measurable disease, and were randomized to receive either H (8 mg/kg → 6 mg/kg q3w) + DOC (100 mg/m² q3w) or H + DOC + BEV (15 mg/kg q3w). H and BEV were given until disease progression; DOC was given for a planned minimum of 6 cycles unless progression or unacceptable toxicity mandated earlier discontinuation. The primary endpoint was progression-free survival (PFS). Additional endpoints included overall survival (OS), overall response rate (ORR; assessed by RECIST 1.0), duration of response, time to treatment failure, safety (NCI CTCAE v3.0 adverse events [AEs] and AEs of special interest for BEV), quality of life (FACT-B), and translational research. The primary analysis of PFS was prespecified after 310 investigator-assessed PFS events. The statistical design provided 90% power to detect a PFS hazard ratio (HR) of 0.69 (median PFS 11→16 months) with α=0.05.

Results: Between Sep 2006 and Feb 2010, 424 patients were enrolled from 60 centers; 421 received treatment. Baseline characteristics were generally well balanced in the H + DOC and H + DOC + BEV arms: median age 55 and 53 years, respectively; visceral metastases 71% and 77%; prior adjuvant H 12% and 13%; disease-free interval <12 months 43% in both arms; measurable disease 85% in both arms. Median follow-up was 26 months in both arms. Efficacy results are summarized in the table.

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>H + DOC</th>
<th>H + DOC + BEV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Investigator assessed</td>
<td>Events, n(%):</td>
<td>Events, n(%):</td>
</tr>
<tr>
<td>HR, unstratified analysis (95% CI)</td>
<td>0.82 (0.65-1.02)</td>
<td>0.76 (0.60-0.96)</td>
</tr>
<tr>
<td>p-value</td>
<td>0.083</td>
<td>0.060</td>
</tr>
<tr>
<td>Median, months (95% CI):</td>
<td>13.7 (11.4-16.3)</td>
<td>16.5 (14.1-19.1)</td>
</tr>
<tr>
<td>HR, stratified analysis (95% CI):</td>
<td>0.76 (0.60-0.96)</td>
<td>0.76 (0.60-0.96)</td>
</tr>
<tr>
<td>p-value</td>
<td>0.083</td>
<td>0.060</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>IRC assessed</th>
<th>Events, n(%):</th>
</tr>
</thead>
<tbody>
<tr>
<td>114 (55)</td>
<td>111 (51)</td>
</tr>
<tr>
<td>HR (95% CI):</td>
<td>0.72 (0.54-0.94)</td>
</tr>
<tr>
<td>p-value</td>
<td>0.061</td>
</tr>
</tbody>
</table>

| Median, months (95% CI): | 13.9 (11.2-16.7) | 16.8 (14.1-19.3) |
| ORR, investigator assessed, %: | 69.9 | 74.3 |
| p-value | 0.0265 | 0.0265 |

| IRC assessed, %: | 65.9 | 76.5 |
| p-value | 0.0045 | 0.0045 |

No new safety signals were observed. The following grade ≥3 AEs were more common in the BEV-containing arm than the H + DOC arm: congestive heart failure (5.1% vs 2.9%, respectively); febrile neutropenia (11.6% vs 8.7%); hypertension (11.6% vs 0.5%). Grade 5 AEs occurred in 1.4% of the H + DOC + BEV arm vs 1.9% of the H + DOC arm.

Conclusions: The addition of BEV to H + DOC improved PFS without reaching statistical significance according to investigator assessment (unstratified HR 0.82; 95% CI 0.65-1.02). The improvement as assessed by Independent Review Committee was statistically significant. Evaluation of biomarkers is ongoing to try to identify those patients who may benefit from first-line BEV-containing therapy for HER2-positive LR/mBC.

S5-1
Neoadjuvant Pertuzumab (P) and Trastuzumab (H): Biomarker Analyses of a 4-Arm Randomized Phase II Study (NeoSphere) in Patients (pts) with HER2-Positive Breast Cancer (BC).
Gianni L, Bianchini G, Kiermaier A, Bianchi G, Im Y-H, Pienkowski T, Roman L, Liu M-C, Tseung L-M, Ratnayeke J, Szado T, Ross GA, Valagussa P. Oncologia Medica, San Raffaele Cancer Center, Milan, Italy; Roche, Basel, Switzerland; Oncologia Medica I, Fondazione IRCCS Istituto Nazionale Tumori, Milan, Italy; Samsung Medical Center, Seoul, Korea; Centrum Onkologii, Warsaw, Poland; Leningrad Regional Oncology Dispansary, St Petersburg, Russian Federation; Koo Foundation Sun Yat-Sen Cancer Center, Taipei, Taiwan; Kapoor V, Veterans General Hospital, National Yang-Ming University, Taipei, Taiwan; Roche Products Limited, Welwyn, United Kingdom; Genentech, South San Francisco; Fondazione Michangelo, Milan, Italy

Background: NeoSphere showed that P with H and docetaxel (T) had a significantly higher pCR rate (45.8%) compared with TH
Materials and methods: Gene expression data on pre-treatment fresh-frozen tumor tissues were collected from a combined cohort of MDACC/I-SPY trial (GSE25055/65), and the XeNA trial (GSE22358). Patients from MDACC/I-SPY were treated with doxorubicin/cyclophosphamide (AC) or 5-fluorouracil/epirubicin or doxorubicin/C (FEC or FAC) sequentially with paclitaxel. All patients from XeNA received capicitabine/docetaxel (and trastuzumab if clinHer2+). Intrinsic subtypes were determined using PAM50. Chi-square test and multivariable logistic regression analysis were used to test significance of association between subtype and pathological complete response (pCR or residual cancer burden [RCB] 0/1) and residual disease (RD or RCB2/3) adjusted with pre-treatment tumor size. Kaplan Meier was used for distant relapse-free survival (DRFS) estimates.

Results: For the MDACC/I-SPY cohort (n=595), pCR rates for intrinsic subtypes were 3% (5/168) for LumA, 16% (14/90) for LumB, 33% (23/69) for HER2-E and 37% (76/208) for Basal-like. Tumors achieving RCB0/1 were significantly associated with better DRFS compared to those tumors with RCB 2/3, even within each intrinsic subtype. The 5-year DRFS for HER2-E with RCB0/1 and RCB2/3 was 100% and 31% (p=0.007), respectively. ClinHer2+ status was also significantly associated with pCR (34% vs. 19%, p=0.016). Strikingly, among the clinHer2+ tumors (n=47), 75% (12/16) of the responders were classified as HER2-E (Table 1A) and those tumors that were clinHer2+/HER2-E had 6 times odds to achieve pCR when compared to clinHer2+/non-HER2-E. In the XeNA trial (n=122), the HER2-E subtype was significantly associated with response, composing 85% (7/8) of the clinHer2+ who achieved a pCR. Finally, clinHer2+/HER2-E tumors were 34 times more likely to achieve pCR than clinHer2+/non-HER2-E tumors (Table 1B).

Conclusion: The sensitivity of clinHer2+ tumors to neoadjuvant anthracycline/taxane-based regimens, and trastuzumab-based chemotherapy is mainly contained within tumors of the HER2-E subtype. Given that this molecular subtype cannot simply be recapitulated using clinical ER and HER2 status, our results highlight the importance of identifying patients with HER2-E tumors as this appears to greatly enrich for responsiveness and treatment benefit.

S5-3
Basement Membrane Localized Tumor Cells Are Protected from HER2-Targeted Therapy In Vivo.

Zoeller JJ, Bronson RT, Gilmer TM, Selfors LM, Lu Y, Mills GB, Brugge JS. Harvard Medical School, Boston, MA; GlaxoSmithKline, Research Triangle Park, NC; UT MD Anderson Cancer Center, Houston, TX

Drug resistance compromises the efficacy of HER2-targeted therapy. Results from our laboratory, and previous reports from others, indicate that tumor cell attachment to basement membrane (BM) and other extracellular matrix (ECM) proteins may confer drug resistance. We have discovered a differential drug response between the outer, matrix-attached cells and inner matrix-deprived cells comprising...
3-dimensional (3D) tumor spheroids grown in reconstituted basement membrane (T. Muranen and J. Brugge, unpublished data). The outer matrix-attached cells are resistant to multiple drug therapies. To address whether these observations are relevant in vivo, we utilized a previously described model of human-in-mouse HER2-positive ductal carcinoma in situ (DCIS), which involves the intraductal transplantation of human HER2-positive SUM225 tumor cells directly into the ductal network of the mouse mammary gland. The intraductal tumors generated are histologically indistinguishable from human DCIS lesions and recapitulate the architecture of the 3D tumor spheroids. The tumors are characterized by organized nests of noninvasive cells confined within a BM surrounded by ECM. These features permit a direct assessment of differential drug response within the tumor. We focused on the tumor cell response to short-term lapatinib monotherapy in vivo. A close examination of the tumor architecture revealed that cells closest to the basement membrane, and nearest to the vasculature, display a striking insensitivity to lapatinib whereas the remainder of the tumor undergoes extensive cell death in response to treatment. Further characterization also revealed that cells closest to the basement membrane largely maintain proliferative capacity despite an overall significant reduction in the total Ki67-positive cell population. These results provide in vivo evidence that basement membrane-attached tumor cells are protected from lapatinib. We confirmed that these cells maintain HER2 status and also observed an overall reduction in pHER2, pAKT and pERK throughout the tumor suggestive of adaptive response mechanisms, which support the proliferation and survival of these cell populations despite inhibition of the HER2 pathway. To further explore potential mechanisms of the adaptive response, we performed reverse phase protein array (RPPA) analysis on protein lysates prepared from tumor biopsy fragments following lapatinib monotherapy. RPPA profile analysis revealed an adaptive response composed of upregulation of multiple RTKs (HER2, IGFI-R) and altered apoptotic protein levels (Bcl-2, Bim, Bcl-xL) in addition to activation of AKT/S6K and ERK/p38 pathway components. These observations suggest that basement membrane-attached tumor cells may escape from lapatinib response via compensatory activation of these survival mechanisms. Each of these components will serve as targets for designing combined therapeutic approaches capable of targeting the protected basement membrane-attached tumor cells. Our results suggest that resistant populations may be a source of residual disease post-therapy, therefore identifying and characterizing these cells will be crucial to the prevention of disease recurrence in the clinic.

S5-4
pCR as a Surrogate in HER2-Positive Patients Treated with Trastuzumab.
Loibl S, von Minckwitz G, Blohmer JU, Costa SD, Eidmann H, Fasching PA, Gerber B, Hanusch C, Hilfirsch J, Huober J, Jackisch C, Kaufmann M, Konecny G, Denkert C, Nekljudova V, Mehta K, Untch M. German Breast Group, Neu-Isenberg; St. Gertrauden Krankenhaus, Berlin; Universitäts-Frauenklinik, Magdeburg; Universitätsklinikum, Kiel; University Hospital, Erlangen; Universitäts-Frauenklinik, Rostock; Klinikum zum Roten Kreuz, München; Henrietten-Stiftung, Hannover; Universitäts-Frauenklinik, Tübingen; Städtische Kliniken, Offenbach; Universitäts-Frauenklinik, Frankfurt; University of California, Los Angeles; Charité, Berlin; Helios Klinikum Berlin-Buch, Berlin

Background: Patients with HER2-positive metastatic disease used to show a more unfavorable prognosis compared to patients with HER2-negative tumors. With the introduction of trastuzumab, patients with HER2-positive metastatic breast cancer show an improved survival compared to patients with HER2-negative tumors (Dawood et al. 2010). So far it has not been shown, if such a switch in prognosis is also achieved in early breast cancer.

Methods: 6377 patients from 7 neoadjuvant German studies with operable or locally advanced, non-metastatic breast cancer were analyzed (for details see von Minckwitz G et al., BCRT 2010). In earlier studies patients (pts) with HER2-positive disease did not receive trastuzumab. Trastuzumab was given in 2 trials parallel to chemotherapy for 12-36 weeks and completed after surgery for up to one year of treatment. All patients with endocrine responsive disease received adjuvant endocrine therapy according to institutional standard. We compared the overall and disease free survival in three subgroups, HER2-negative patients, HER2-positive w/o trastuzumab and HER2-positive patients with trastuzumab according to pCR defined as ypT0, ypN0.

Results: 6377 patients were evaluable. During a median follow up of 46.3 (0-127) months and observation of 22,869 patient years, 1466 (23%) relapses and 775 (12.2%) deaths were observed. 3060 had HER2-negative disease, 665 patients had HER2-positive disease w/o trastuzumab and 662 patients with HER2-positive disease received trastuzumab. No data on HER2 status were available in 1990 (31.2%) patients as measurement of HER2 was only implemented in the study procedures since 2001. Median age of patients at time of study entry was 50.1 (21-81) years. Median tumor size was 4.0 (range 1.2 – 33.0) cm. Overall 15% (955) of patients had a pCR. The pCR rate in HER2-positive pts was 24% in those with trastuzumab and 15.8% in those without. There was no difference in overall DFS in the three groups achieving a pCR (log rank p=0.251). There was a strong trend towards a better OS in pCR HER2+pts being treated with trastuzumab (overall log rank p= 0.067). Cox regression analysis revealed HER2 positive patients with trastuzumab had a better OS than HER2-positive pts w/o trastuzumab (HR: 7.44; 95%CI [0.92-60.1; p=0.06) and HER2negative pts (HR: 3.86; 95% CI [0.5-29.41], p=0.19).

However, for non-pCR pts, DFS was significantly inferior for pts treated with trastuzumab compared to patients without trastuzumab (HR: 0.81, 95% CI [0.63-1.04), p=0.102) or pts with HER2-negative tumors (HR: 0.75; 95% CI [0.61-0.92] p=0.006.(overall log-rank p=0.022). However, OS was not significantly different between the three groups of non-pCR pts.

Conclusion: Patients with HER2-positive primary breast cancer treated with trastuzumab achieve a higher pCR rate. This higher absolute number of pCRs in trastuzumab-treated patients lead to a DFS at least as good as that of HER2-positive not trastuzumab treated and HER2-negative patients and OS even tended to be superior to the other two groups. This supports that pCR can be considered as a surrogate marker in HER2-positive disease. However, HER2-positive, trastuzumab-treated patients without a pCR are at high risk of relapse and are at high medical need for new treatment options.
**S5-5**

A Phase III, Randomized, Double-Blind, Placebo-Controlled Registration Trial To Evaluate the Efficacy and Safety of Pertuzumab + Trastuzumab + Docetaxel vs. Placebo + Trastuzumab + Docetaxel in Patients with Previously Untreated HER2-Positive Metastatic Breast Cancer (CLEOPATRA).

Baselga J, Kim S-B, Im S-A, Hegg R, Im Y-H, Roman L, Pedrini JL, Cortés J, Knott A, Clark E, Ross GA, Swain SM. Massachusetts General Hospital Cancer Center; Boston, MA; Asan Medical Center; University of Ulsan, College of Medicine, Seoul, Korea; Seoul National University College of Medicine, Seoul, Korea; Hospital Pórola Byington, São Paulo, Brazil; Samsung Medical Center; Sungkyunkwan University School of Medicine, Seoul, Korea; Leningrad Regional Oncology Dispensary, St Petersburg, Russian Federation; CPMEC-Mastology Unit of Concópia Hospital, Porto Alegre, Brazil; Vall d’Hebron University Hospital, Barcelona, Spain; Roche Products Limited, Welwyn, United Kingdom; Washington Cancer Institute, Washington Hospital Center; Washington, DC

**Background:**

Pertuzumab (P) is a fully humanized investigational monoclonal antibody that binds to human epidermal growth factor receptor 2 (HER2), preventing dimerization of HER2 with other HER family members and inducing antibody-dependent cell-mediated cytotoxicity. Its mechanisms of action are complementary to those of the anti-HER2 antibody trastuzumab (H) and the two antibodies combined have superior activity compared with either antibody alone in preclinical and clinical studies. In patients with advanced disease, P in combination with H has been shown to be active in patients whose disease has progressed while on H therapy (Baselga et al, *J Clin Oncol* 2010). Furthermore, P has been shown to improve the activity of H and docetaxel (T) in a randomized neoadjuvant study (Gianni et al, *SABCS* 2010, S3-2). No increase in overall toxicity and, in particular, no increase in cardiac events was observed with the addition of P to H and HT regimens.

**Methods:**

In this double-blind Phase III study patients with centrally confirmed HER2-positive metastatic or locally recurrent, unresectable breast cancer were randomized to receive either placebo+H+T or P+H+T. Patients could have received one prior hormonal treatment for metastatic breast cancer and/or prior systemic neoadjuvant or adjuvant therapy including prior H and T. Patients had to have a baseline left ventricular ejection fraction ≥50% and no history of declines to <50% during or after prior H therapy.

Study medication was as follows: P 840 mg loading dose followed by 420 mg q3w; H 8 mg/kg loading dose followed by 6 mg/kg q3w; T 75 mg/m2 q3w (with subsequent dose escalation to 100 mg/m2 if 75 mg/m2 was well tolerated). Patients were recommended to receive at least 6 cycles of T. In the case of chemotherapy discontinuation due to cumulative toxicity, antibody therapy was continued until disease progression, unacceptable toxicity, or withdrawal of consent. Patients were stratified according to region and prior treatment status (adjuvant therapy or de novo metastatic breast cancer).

The primary endpoint for the study was progression-free survival (PFS) as determined by independent review. The primary analysis was planned to take place when approximately 381 independently confirmed PFS events had occurred. This would provide 80% power to detect a 33% improvement in PFS (HR=0.75) at the two-sided significance level of 5%. Secondary endpoints included overall survival, investigator-determined PFS, overall response rate, duration of response, safety, and quality of life.

Patient safety was monitored throughout the study by an independent data monitoring committee and a cardiac review committee.

This study is registered at ClinicalTrials.gov: NCT00567190.

**Results:**

808 patients were recruited for February 2008 and July 2010. The required number of PFS events for analysis of the primary endpoint has been reached and independent assessment PFS is currently being performed. Results of the primary analysis of efficacy and safety will be presented.

**S5-6**

Neoadjuvant Pertuzumab and Trastuzumab Concurrent or Sequential with an Anthracycline-Containing or Concurrent with an Anthracycline-Free Standard Regimen: A Randomized Phase II Study (TRYPHAENA).

Schneeweiss A, Chia S, Hickish T, Harvey V, Eniu A, Hegg R, Tausch C, Seo J-H, Tsai Y-F, Ackrill A, Ross G, Cortès J. National Center for Tumor Diseases, University Hospital, Heidelberg, Germany; British Columbia Cancer Agency - Vancouver Centre, University of British Columbia, Vancouver, Canada; Royal Bournemouth Hospital, Bournemouth University, Bournemouth, United Kingdom; Regional Cancer and Blood Centre, Auckland City Hospital, Auckland, New Zealand; Cancer Institute “I. Chiricuta”, Cluj-Napoca, Romania; Hospital Pórola Byington, São Paulo, Brazil; Breast-Center, Zürich, Switzerland; Department of Internal Medicine, Korea University Guro Hospital, Guro, Korea; Taipey Veterans General Hospital, Department of Surgery, Taipei, Taiwan; Roche Products Limited, Welwyn, United Kingdom; Vall d’Hebron University Hospital, Barcelona, Spain

**Background:**

Pertuzumab (P) is a fully humanized investigational monoclonal antibody that binds to human epidermal growth factor receptor 2 (HER2), preventing homo- and heterodimerization of HER2 and other HER family members and inducing antibody-dependent cell-mediated cytotoxicity. The NeoSphere study demonstrated significantly increased antitumor activity in the neoadjuvant setting for the combination of P and trastuzumab (H) plus docetaxel (T) compared with HT alone (Gianni et al, *SABCS* 2010), consistent with the hypothesis of complementary mechanisms of action of P and H. No increase in cardiac risk was observed with the addition of P to H and HT regimens. Anthracyclines have an important role in the treatment of breast cancer and may be especially efficacious in HER2-positive disease. In the TRYPHAENA study, P and H are being administered either sequentially or concurrently with an anthracycline-containing or concurrently with an anthracycline-free standard regimen in order to establish the tolerability profile of these regimens for testing in the adjuvant setting.

**Methods:**

Patients with centrally confirmed stage II or III (including locally advanced and inflammatory disease) HER2-positive breast cancer (defined as centrally confirmed HER2 IHC 3+ or FISH/CISH+) were randomized to receive:

<table>
<thead>
<tr>
<th>Arm</th>
<th>Neoadjuvant therapy q3w x 6 cycles</th>
<th>Adjacent therapy</th>
<th>H q3w x 17/20cycles</th>
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<tbody>
<tr>
<td>A</td>
<td>Pertuzumab + trastuzumab + docetaxel (PTX) + docetaxel (T) followed by H and T x 17/20cycles</td>
<td>HA 17/20</td>
<td>H + PA + H x 17</td>
</tr>
<tr>
<td>B</td>
<td>Sequential (n=75)</td>
<td>FEC x 3, followed by TPA x 3</td>
<td>H x 20</td>
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<tr>
<td>C</td>
<td>Anthracycline-free (n=75)</td>
<td>Carboplatin + TPX x 6</td>
<td>H x 17</td>
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Study medication: P 840 mg loading dose and 420 mg maintenance; H 8 mg/kg loading dose and 6 mg/kg maintenance; T was given at 75 mg/m2 with dose escalation to 100 mg/m2 if the starting dose was well tolerated (no dose escalation in Arm C); FEC (5-fluorouracil 500 mg/m2, epirubicin 100 mg/m2, and cyclophosphamide 600 mg/m2) and carboplatin AUC 6. All treatments were given intravenously q3w. The study is ongoing. All patients will receive H for 1 year. The primary endpoint is assessment of the safety and tolerability of neoadjuvant treatment. During neoadjuvant treatment, left ventricular ejection fraction is assessed at Cycles 2, 4, and 6 by echocardiogram or multiple-gated acquisition. Safety data are supervised by a data

**Cancer Res; 71(24 Suppl.) December 15, 2011 112s Cancer Research**
and safety monitoring board. The key secondary endpoint is the rate of pathological complete response (pCR) defined as absence of invasive disease in the breast at surgery. Tissue specimens at baseline and post-surgery were required for all patients. This study is registered at ClinicalTrials.gov: NCT00976989.

**Results:** 225 patients have been recruited. No arm has required modification due to safety concerns or to excessive incidence of disease progression. Analysis of the study results is anticipated in Q3 2011. Safety/tolerability and efficacy (pCR) data will be presented.

**SS-7**


Martín M, Bonnetterre J, Geyer, Jr CE, Ito Y, Ro J, Lang I, Kim S-B, Germa C, Vernette J, Vo Van ML, Wang K, Wang K, Awada A. Hospital Universitario Gregorio Marañón, Madrid, Spain; Centre Oscar Lambret, Lille, France; Allegheny Cancer Center, Pittsburgh, PA; The Cancer Institute Hospital of JFCR, Tokyo, Japan; National Cancer Center, Goyang, Korea; National Institute of Oncology, Budapest, Hungary; Asan Medical Center, Seoul, Korea; Pfizer Global Research and Development, Paris, France; Pfizer Inc, Collegeville, PA; Bristol Meyers Squibb, Princeton, NJ; Jules Bordet Institute, Brussels, Belgium

**Background:** Neratinib (N), an irreversible pan-tyrosine kinase inhibitor (TKI), with activity against HER1, -2 and -4, has shown antitumor activity in patients (pts) with HER2+ breast cancer (BC). Lapatinib (L), a reversible HER1 and -2 TKI is approved in combination with capecitabine (C) for treatment of pts with HER2+ advanced or metastatic BC who had prior therapy.

**Materials and Methods:** This phase 2, randomized, open-label study evaluated safety and efficacy of N 240 mg/day vs L 1,250 mg/day plus C 2,000 mg/m²/day (14 days/21 day cycle) in pts with HER2+ locally advanced or metastatic BC. Eligible pts had: ≤2 prior trastuzumab regimens, prior taxane treatment, and no prior anthracycline treatment with cumulative dose >400 mg/m² doxorubicin, >800 mg/m² epirubicin, or >equivalent dose of other anthracycline. Primary endpoint was progression-free survival (PFS; investigator-assessed); secondary endpoints included safety, overall survival (OS), objective response rate (ORR), and clinical benefit rate (CBR; % pts with complete response, partial response, or stable disease ≥24 wks). Tumor assessments were every 6 wks for the first 48 wks, then every 12 wks until progressive disease (PD; RECIST 1.0) or initiation of new anticancer therapy.

**Results:** Overall, 117 pts were randomized to N and 116 to L plus C (LC). Mean age (SD; range) was 53.9 y (10.3; 28-79); 60% were White, 34% Asian, and 6% other. Prior treatments included: trastuzumab (229 pts: 168 metastatic or locally advanced, 51 adjuvant, 10 neoadjuvant), taxanes (230 pts), and anthracycline (156 pts). Median treatment duration (range) was 126.5 days (1-636) for N and 201 days (13-622) for LC. Median relative dose intensity (actual/expected exposure) for N was 100%. As of data cutoff, 84% had discontinued treatment; 65% from PD (N 63%, LC 67%), 9% for adverse events (AEs; N 7%, LC 11%). In the ITT cohort, for N and LC, respectively, median PFS (95% CI) was 4.5 mo (3.1-5.7) and 6.8 mo (5.9-8.2); P = 0.091; hazard ratio = 1.3 (95% CI, 1.0-1.8); median OS (95% CI) was 19.4 mo (19.4-22.2; 41 deaths) and 19.0 mo (16.9-NA; 35 deaths; P = 0.180); ORR (95% CI) was 29% (21-38) and 41% (32-50; P = 0.067); CBR (95% CI) was 44% (35-54) and 64% (54-73; P = 0.003). Most common drug-related treatment-emergent AEs (TEAEs; any grade) were diarrhea (N 84%, LC 67%), nausea (34%, 38%), palmar-plantar erythrodysesthesia (PPE; 5%, 63%), and rash (18%, 34%); for grade ≥3, diarrhea (28%, 10%) and PPE (0, 14%). Dose reductions/discontinuations from diarrhea occurred in 13/3 pts on N, and 15/7 pts on LC. Study deaths for N and LC, respectively, included: 36 pts (31%) and 32 pts (28%) from PD; 5 pts (4%) and 3 pts (3%) from AEs unrelated to study drug.

**Discussion:** In this setting of pts less heavily pre-treated than in the pivotal LC trial, single agent N demonstrated high anti-tumor activity (ORR 29%), confirming results from prior N phase 2 trials. N alone did not appear to be as effective as LC. No unexpected TEAEs were observed; N was well tolerated in pts with HER2+ locally advanced or metastatic BC; while diarrhea was more frequent on N than LC, it was manageable with antiidiarrheals and did not lead to more treatment discontinuations.

**S6-1**

Menopausal-Specific and Health-Related Qualities of Life among Post-Menopausal Women Taking Exemestane for Prevention of Breast Cancer: Results from the NCIC CTG MAP.3 Placebo-Controlled Randomized Controlled Trial.

Maunsell E, Richardson H, Ingle JN, Ales-Martinez JE, Chlebowski RT, Fabian CJ, Sarto GE, Garber JE, Pujol P, Hiltz A, Tu D, Goss PE. Université Laval, Quebec City, QC, Canada; Queen’s University, Kingston, ON, Canada; Mayo Clinic, Rochester, MN; Hospital Nira Sra Sonsoles, Avila, Spain; Los Angeles Biomedical Research Institute, Torrance, CA; University of Kansas Medical Center, Westwood, KS; Center for Women’s Health and Health Research, Madison, WI; Dana Farber Cancer Institute, Boston, MA; CHU-Hopital Arnaud de Villeneuve, Montpellier, France; Massachusetts General Hospital Cancer Center, Boston, MA

**Background:** Exemestane, a steroidal aromatase inhibitor, reduced the incidence of invasive breast cancers by 65% among 4560 post-menopausal randomized to exemestane or placebo for 5 years on MAP.3. Differences in quality of life (QOL) were judged to be minimal, but only summary information was reported. **Purpose:** To provide more detailed information about effects of exemestane on menopause-specific and health-related qualities of life. **Method:** Participation in quality of life assessment was an eligibility criterion. Menopause-specific and health-related qualities of life were assessed using the MENQOL (4 scales; physical, vasomotor, psychosocial, sexual) and SF-36 (8 scales; physical health, role function – physical, bodily pain, general health, vitality, social function, role function – emotional, mental health, and 2 summary scales) instruments, respectively at baseline, 6 months and then yearly after randomization. Compliance with QOL questionnaire completion at each follow-up visit ranged from 93-98%, and did not differ by group. Change scores for each MENQOL and SF-36 scale, calculated for each assessment time relative to baseline, were compared using the Wilcoxon Rank-Sum test. Summary scores were used to summarize the QOL scores observed at each time point for each SF-36 dimension and overall mental (MCS) and physical component summaries (PCS) and MENQOL domains. Clinically important worsening of MENQOL change scores was defined as an increase of ≥0.5/8 points. SF-36 change scores were considered worsened if scores decreased by ≥5 points from baseline. **Results:** Both groups were balanced on scores for MENQOL and SF-36 at baseline. Median follow-up was 35 months and the proportion of women who stopped study medication early for toxicity reasons was 15% in the exemestane arm and 11% in the placebo arm. There was a statistically significant difference in change scores for vasomotor symptoms among women on exemestane during the first 4 years (p-values <0.01), compared to
placebo. However, no between-group differences in vasomotor change met the criterion for clinical importance. Women on exemestane had statistically poorer sexual functioning (mean change = -0.02, SD=1.37) compared to placebo (mean change = -0.12, SD=1.32) during the first 6 months on study (p-value = 0.03) but the differences were not statistically significant thereafter or clinically important at any time. Among the 8 SF-36 scales, only bodily pain was statistically different between exemestane and placebo for the first 24 months on study medication (p-value <0.01), but no between-group difference in change scores exceeded 5 points. Overall SF-36 PCS and MCS assessing changes in overall physical and mental health-related QOL did not differ significantly by group at any assessment. Conclusion: Our assessment that early differences in vasomotor symptoms and pain were probably not clinically important is supported by the observation of no between-group differences when overall physical and mental health-related QOL changes were compared. Exemestane does not appear to have a major negative impact on the quality of life among these women.

S6-2
Patient-Reported Predictors of Early Treatment Discontinuation: NCIC JMA.27/E1Z03 Quality of Life Study of Postmenopausal Women with Primary Breast Cancer Randomized to Exemestane or Anastrozole.
Wagner LI, Zhao F, Chapman J-AW, Cella D, Shepherd LE, Sledge GW, Goss PE. Northwestern University Feinberg School of Medicine, Chicago, IL; Dana-Farber Cancer Institute, Boston, MA; NCIC Clinical Trials Group, Queen’s University, Kingston, ON, Canada; Indiana University Simon Cancer Center, Indianapolis, IN; Massachusetts General Hospital, Boston, MA
Background: Patient-reported outcomes (PRO) assess symptom burden and health-related quality of life (HRQL) more accurately than clinical report. We previously found that treatment-emergent adverse events (CTCAE V3.0) did not impact efficacy in the main NCIC MA.27 trial, although many women discontinued treatment early. The purpose of this substudy was to obtain PROs in MA.27/E1Z03 participants to evaluate treatment-related side effects and HRQL between exemestane and anastrozole and to identify predictors of treatment discontinuation.

Methods: The Eastern Cooperative Oncology Group trial (E1Z03) assessed PROs in a sample of MA.27 patients (N=686; 99.3% participation rate). Participants completed the 56-item Functional Assessment of Cancer Therapy–Endocrine Symptoms (FACT-ES) pre-treatment and at months 3, 6, 12 and 24 to assess breast-cancer specific concerns, side effects of hormonal treatments and HRQL. We used 1) Wilcoxon rank sum test to compare treatment-related symptoms and HRQL between trial arms; 3) linear regression to examine the effects of symptoms on HRQL and 4) adjusted Cox regression to assess predictors of treatment duration.

Results: Participants included 371 women randomized to anastrozole and 315 randomized to exemestane. Demographic and disease characteristics were balanced between trial arms. Participants were a mean age of 65.6 yrs (SD=9.2), White (95.9%), ECOG PS of 0 (87.2%), stage T1 (75.5%) or T2 (22.7%), and stage N0 (73.9%) or N1 (16.3%). Prior treatments included partial mastectomy (65.7%), chemotherapy (27.8%) and radiotherapy (51.7%). Treatment-related symptoms measured by 23 items from the FACT-ES did not differ between treatment arms at months 3, 6, 12 and 24 and the timeline change of treatment-related symptoms was similar between treatment arms (p = ns). HRQL was significantly impacted by decreased libido, weight gain, feeling bloated, breast sensitivity, mood swings, irritability, join pain, nausea and bother by treatment side effects (p < 0.001). 248 participants were off-treatment by 4.1 yrs. A Cox model adjusted for other symptoms, demographic and disease characteristics indicated the hazard of discontinuing treatment early increased by 29% when the severity of being bothered by side effects at baseline increased by 1 point (HR=1.29, 95% CI:1.09-1.54). At baseline, patients with prior treatments or taking more medicines reported more bother by side effects (p < 0.001). Increased joint pain in the first 3 months after treatment was also associated with increased hazard of discontinuing treatment early (HR=1.13, 95% CI: 1.01-1.28).

Conclusions: PRO assessment indicated comparable symptom burden and HRQL among postmenopausal women randomized to anastrozole or exemestane, with increasing symptom burden over time negatively affecting HRQL. Patients who 1) initiate an aromatase inhibitor (AI) with bother by side effects from prior treatment and concomitant medications and 2) experience increased joint pain during the first 3 months of AI therapy are at risk for early discontinuation of AI therapy.

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S6-3
Influence of Hospital Factors, Physician Factors and Type of Health Insurance on Receipt of Immediate Postmastectomy Reconstruction in Young Women with Breast Cancer.
Hershcman DL, Neugut AI, Richards CA, Kalinsky K, Charles AS, Wright JD. Columbia University, New York, NY; Mailman School of Public Health, New York, NY
Objective
For women with breast cancer who choose mastectomy, breast reconstruction is known to offer a cosmetic and psychological advantage. Despite this, only a minority of patients undergo post mastectomy reconstruction. Little is known about factors that influence reconstruction in younger women who undergo mastectomy. We evaluated the association of demographic, hospital, physician and insurance factors with receipt of immediate breast reconstruction.

Methods
We used the Perspective database to identify women who underwent a mastectomy for breast cancer from 2000-2010. Perspective is a voluntary, fee-supported database that samples more than 500 acute-care hospitals throughout the United States that contribute data on inpatient hospital admissions. ICD-9 procedure codes were used to identify women who underwent reconstruction at the time of mastectomy. Differences in reconstruction rates over time were examined by age, race, and type of insurance (commercial, Medicaid, Medicare and self-pay). Logistic regression analysis was used to determine factors predictive of immediate breast reconstruction after mastectomy. Additional analyses were done on the population of younger women (<50 years of age).

Results
We identified 106,988 women with breast cancer who underwent mastectomy, and of these, 24,150 (22.6%) underwent immediate reconstruction. From 2000 to 2010, reconstruction rates increased from 15% to 33.3%, increasing the most for women with commercial insurance (25.3% to 54.6%) and women under the age of 50 (29% to 60%); for women under the age of 50 with commercial insurance the rate in 2010 was 67.5%. Multivariable analysis found that reconstruction was significantly less likely with increasing age, black race (OR=0.66), rural hospital location (OR=0.48), non-teaching hospital (OR=0.82) and >2 co-morbid conditions (OR=0.72). Odds of reconstruction increased with commercial (OR=3.0) and public
(OR=1.6) insurance (compared to self-pay), bilateral mastectomies (OR=2.5), being single (OR=1.09) and increased hospital volume (OR=1.94). No association was found with breast surgeon volume. Similar associations were seen in the subgroup of women <50 years of age. Prolonged length of stay was greater for women undergoing reconstruction (29.2% vs. 18.5%, p<0.0001); however, in-hospital complication rates were similar at 5.6% and 5.3%, respectively.

**Conclusion**
Despite its benefits, less than one-third of all women, and half of women under the age of 50, received post mastectomy reconstruction in 2010. Insurance status was one of the largest predictors of immediate reconstruction, and its influence has increased over time. Public policy should ensure that access to reconstructive surgery is available to all women, regardless of insurance type.

**S6-4**
**Protein Kinase Mutation Patterns in Human Breast Cancer.**

**Paszta L, Qi Y, Shi W, Liu C-G, Wang B, Liu X, Booser D, Esteva FJ, Symmans F, Hortobagyi GN. UT MD Anderson Cancer Center; Houston, TX**

**Background:** We performed next generation targeted sequencing of all known human protein kinases (n=530 genes) and 56 additional cancer related genes (BRCA1,2, p53, PTEN, etc.) in 112 samples to assess the kinase mutation landscape of breast cancer. Gene expression profiling was also performed on RNA from each specimen and DNA copy number variations were assessed in a subset of 59 cases using array CGH. The three types of genomic data were mapped to canonical biological pathways to identify frequently genomically disturbed pathways in these cancers. **Methods:** DNA and RNA extracted from fine needle biopsies of 92 breast cancers were analyzed, 20 samples were sequenced in duplicates or triplicates to assess technical variation of the results. Targeted sequencing was performed with Agilent SureSelect Human Kinome kit and the SOLiD sequencing platform. Gene expression profiling and array CGH were performed with Affymetrix U133A chips and Agilent 244K CGH array. Sequence data was mapped to hg19, functional impact scores were calculated with SWIFT, canonical pathways were obtained from the Broad Institute.

**Results:** 0.1% of the entire genome was sequenced and >80% of target base pairs had >20-fold coverage. The mean number of single nucleic acid variants (SNV) and indels per sample was 1043 (range: 493-1583, about 60% homozygous) and 159 (range: 75-269) respectively, 97% of SNVs and 78% of indels were already represented in dbSNP or COSMIC data bases. About 20% of SNVs were predicted to alter kinase or other biological function. The mean number of functionally high impact SNVs was 28 per sample (range 11-47). In addition to known p53 and PI3K mutations we detected frequent mutations in BRCA1 (20%) and observed several predicted high impact SNVs in HER2 (20%) as well as in many MAPK family enzymes. Not all SNVs were distributed equally across disease subsets, SNVs in ULK4, BMP2K, PALB2, ALPK3 were more frequent in triple negative cancers (TNBC) whereas SNVs in EPHA2 was more common in ER+ cancers. Among TNBC, those with residual cancer after neo-adjuvant chemotherapy (n=22) had significantly higher rates of SNVs in HUNK, TRPM7, NEK1 and HER3 compared to cases with pathologic complete response (n=25). When high impact SNVs, DNA copy number alterations and gene over-expression (relative to normal breast n=45) observed in individual cases were mapped to biological pathways a complex network of anomalies emerged for each case. **Conclusion:** We observed several known mutations in cancer genes and also detected many SNVs in important regulatory genes that were previously described as functional, germ-line variants with experimentally validated or suspected impact on protein function. Individual cancers have unique combinations of these events. This suggest that cancers arise in the context of complex genomic “germ line noise” which may determine which types of somatic events can or cannot “drive” individual cancers. The data also suggest therapeutic hypotheses about what biological pathways should be targeted in individuals and in subsets of cancers.

**S6-5**
**Obesity Drives Epithelial-to-Mesenchymal Transition and Tumor Progression in a Novel Claudin-Low Mammary Cancer Model.**

**Duplak SM, Chiao LJ, Nogueria L, Usary J, Perou CM, Varticovski L, Hursting SD. University of Texas, Austin, TX; University of North Carolina, Chapel Hill, NC; National Cancer Institute, Bethesda, MD; University of Texas M.D. Anderson Cancer Center; Smithville, TX**

Background: Epidemiological evidence suggests a potential role of obesity in regulating clinical subtype, differentiation status, and prognosis of breast cancer. Specifically, an elevated waist-hip ratio is associated with increased risk and progression of basal-like breast cancer, an aggressive form characterized by heterogeneous tumors typically enriched in a putative tumor initiating cell (TIC) population. However, the exact mechanism of obesity-driven tumor progression remains unknown. Therefore, we hypothesized that obesity regulates a plastic population of multipotent malignant cells.

**Materials, Methods, and Results:** To test this hypothesis, we generated and characterized two distinct murine mammary tumor cell lines derived from MMTV-Wnt-1 transgenic mice, designated M-Wnt and E-Wnt. M-Wnt cells displayed a mesenchymal morphology while E-Wnt cells had an epithelial morphology. M-Wnt cells harbored a large CD44+/CD24- putative TIC population (62% +/- 7.8), had significant mammosphere forming capacity (>30%) of cells form mammospheres, p<0.0001), and increased ALDH activity (7% are ALDH+, p=0.0004). M-Wnt cells have increased migration (scratch assay) and invasion in vitro (226-fold higher after 30h, p<0.0001). As few as 50 unsorted M-Wnt cells, injected into C57BL/6 mice were capable of forming a tumor. Microarray analysis revealed that M-Wnt cells display gene expression profiles virtually identical to human Claudin-low breast tumors, while E-Wnt cells clustered with basal-like tumors. EMt and stem cell gene expression patterns of M-Wnt cells were maintained in vivo, including decreased E-cadherin and increased N-cadherin, fibronectin, vimentin, SNAI1, TWIST, SLUG, FOXC2, and TGF-β (p<0.05 for all). Using these cell lines in vivo, we tested the hypothesis that energy balance modulation, through diet-induced obesity (DIO) and calorie restriction (CR), regulates the TIC population. We found that M-Wnt tumors, transplanted into ovariectomized syngeneic female C57BL/6 mice, grew at significantly different growth rates depending on the diet treatment (DIO > Control, p=0.011; CR < Control, p=0.012), while E-Wnt tumors were only affected by CR (CR < Control p=0.001; no difference between DIO and Control tumors). DIO enhanced M-Wnt tumor progression, adipocyte infiltration, and central necrosis and drove EMT (decreased E-Cadherin and increased fibronectin and N-Cadherin expression) through modulation of key TIC associated genes, including increased TGF-β, SNAI1, FOXC2, and Oct4 (p<0.05 for all).

**Discussion:** In conclusion, we found that the mesenchymal M-Wnt cell line is highly responsive to changes in dietary energy balance status relative to the E-Wnt differentiated epithelial cell line, which demonstrates that clinical subtypes of breast cancer are differentially regulated by energy balance modulation. Additionally, our data demonstrates, for the first time, that energy balance modulation (ie, CR and obesity) directly regulates EMT, in response to local upregulation of TGF-β signaling.
S6-6
Expression of Key Estrogen-Regulated Genes (ERGs) Differ Substantially across the Menstrual Cycle in ER+ Breast Tumours.

Haynes BP, Vade B, A’Hern R, Smith IE, Dowsett M, Galimberti V, Rotmensz N, Gibelli B. Royal Marsden Hospital, London, United Kingdom; Institute of Cancer Research, Sutton, Surrey, United Kingdom; European Institute of Oncology, Milan, Italy

Aim: To determine whether there are substantial changes in the expression of ERGs in estrogen receptor positive (ER+) breast cancer through the menstrual cycle.

Background: Plasma levels of estradiol (E2) vary from c.100pM to c.1000pM and progesterone levels from <3nM to >50nM through the menstrual cycle. The changes in E2 are proportionally similar to those that occur in postmenopausal women treated with an aromatase inhibitor which lead to profound changes in the expression of ERGs. However, there are only inconsistent data on whether the cyclical changes in hormone levels during the menstrual cycle affect gene expression in ER+ tumours.

Methods: 173 paraffin-embedded ER+ breast carcinomas were analysed from premenopausal patients in which day of menstrual cycle and hormonal data were recorded at the time of surgery. Patients were ascribed to one of 3 pre-defined time windows of the menstrual cycle: window 1: days 27-35 + 1-6 (low circulating E2 and progesterone); window 2: days 7-16 (high E2 and low progesterone); window 3: days 17-26 (moderate E2 and high progesterone). RNA was extracted (RecoverAll; Ambion) and QPCR used to measure expression of ESR1, 4 ERGs (TFF1, PGR, GREB1 and PDZK1) and 3 housekeeping genes.

Results: ESR1 expression did not differ significantly across the menstrual cycle but there was strong evidence of differences in the ERGs (Kruskal-Wallis; p≤0.0015 to 0.093). Gene expression levels of the 4 ERGs were 51-109% higher in window 2 than window 1 (see table) and this was significant for PGR, GREB1 and TFF1 and approached significance for PDZK1. The expression of all the ERGs was lower (14-59%) in window 3 compared to window 2 (significant for TFF1). PGR and GREB1 expression was significantly different (30-40%) in window 3 compared to window 1.

<table>
<thead>
<tr>
<th>% difference in median expression for ER+ tumours (Mann-Whitney p)</th>
<th>PGR</th>
<th>GREB1</th>
<th>TFF1</th>
<th>PDZK1</th>
<th>AvERG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Difference window 1 and 2</td>
<td>109.5 (p=0.0041)</td>
<td>51.4 (p=0.0006)</td>
<td>88.8 (p=0.037)</td>
<td>97.5 (p=0.092)</td>
<td>63.2 (p=0.0002)</td>
</tr>
<tr>
<td>Difference window 2 and 3</td>
<td>-33.1 (p=0.20)</td>
<td>-13.9 (p=0.20)</td>
<td>-52.4 (p=0.028)</td>
<td>-58.8 (p=0.061)</td>
<td>-30.5 (p=0.007)</td>
</tr>
<tr>
<td>Difference window 1 and 3</td>
<td>+0.42 (p=0.049)</td>
<td>+10.1 (p=0.02)</td>
<td>-10.1 (p=0.20)</td>
<td>-18.5 (p=0.20)</td>
<td>-13.4 (p=0.17)</td>
</tr>
</tbody>
</table>

The average expression of these 4 ERGs gives the AvERG, a previously defined index of estrogen responsiveness. This showed similar changes to the individual ERGs but the reduced variability of the AvERG led to even greater statistical significance. A control set of ER-tumours (n=83) showed very low expression of the ERGs and no cyclical changes.

Discussion: These data reveal significant changes in breast tumour biology across the menstrual cycle. As these changes are likely to occur only in tumours that are estrogen-dependent their measurement may potentially be developed as a test of a tumour’s likely response to estrogen deprivation. Several ERGs are present in currently used molecular profiling tests; variable expression of these markers through the menstrual cycle may affect the interpretation of the tests.

The addition of dasatinib to fulvestrant would increase progression-free survival (PFS) over fulvestrant alone in MBC. Methods: This is a randomized phase II study including patients with evaluable or measurable ER+ MBC whose disease had progressed while on adjuvant or metastatic NSAI. Patients were stratified to ≤ 2 yrs vs > 2 yrs from initial breast cancer diagnosis to first diagnosis of metastatic disease and randomly assigned 1:1 to Arm 1 (loading dose of fulvestrant 500 mg followed by 250 mg IM and dasatinib 100 mg PO QD) or Arm 2 (single-agent fulvestrant 250 mg IM). Following FDA approval in 2010 of the 500 mg dose, a loading dose (500 mg) of fulvestrant on Day 1 was followed by 500 mg on Day 15 of Cycle 1 and on Day 1 of each subsequent cycle (n=40 patients received the higher dose). Arm 2 patients who progressed while on fulvestrant could cross over to receive fulvestrant plus dasatinib. The primary objective was median PFS. Secondary objectives included overall survival (OS), overall response rate (ORR), and safety.

Results: The study included 99 patients, 50 in Arm 1 (combination) and 49 in Arm 2 (fulvestrant alone). Results for Arm 1 and Arm 2, respectively, were: median PFS 6.0 and 5.3 months; median OS 17.0 and 21.7 months; and clinical benefit rates (CBR) (PR+SD≥6 months) were 14 (28.0%, 95%CI=16.2%-42.5%) and 16 (32.7%, 95%CI=19.9%-47.5%) months. In Arm 1 and Arm 2, 24% and 8% developed grade 1/2 pleural effusion; 34% and 10% developed grade 1/2 diarrhea, 40% and 6% developed grade 1/2 nausea, and 30% and 18% developed grade 1/2 fatigue. Six patients on Arm 1 stopped dasatinib due to AEs, mainly pleural effusion and dyspnea. Eighteen (37%) Arm 2 patients crossed over to receive combined fulvestrant/dasatinib at disease progression on fulvestrant. Median PFS for crossover patients was 3.8 months, with the longest duration of treatment being 12 and 15 months in 2 patients.

Conclusions: The addition of dasatinib to fulvestrant in ER+ postmenopausal MBC patients whose disease had progressed through an NSAI did not improve PFS, CBR, or OS. The combination of fulvestrant and dasatinib in patients with ER+ MBC was well.
tolerated. Formalin-fixed paraffin-embedded archival BC tissue is being analyzed for SRC-related signature to determine whether a subset of patients benefit from dasatinib.

**PD01-02**

**Randomized Phase II Study of Dasatinib vs Placebo in Addition to Exemestane in Advanced ER/PR-Positive Breast Cancer [BMS CA180-261 Study].**

Llombart A, Ravaiolli A, Straus L, Sy O, Abrahao F, Geese WJ, Lortholary A, Rea D, Ro J-S, Sohn J, Kim S-B, Curigliano G. Hospital Universitari Arnau De Vilanova, Lleida, Spain; Ospedale degli Infermi di Rimini, Rimini, Italy; Bristol-Myers Squibb, Wallingford, CT; Centre Catherine De Sienne, Nantes, France; City Hospital, Birmingham, United Kingdom; National Cancer Center, Gyeonggi-Do, Korea; Yonsei Cancer Center, Seoul, Korea; Asan Medical Center, Seoul, Korea; Instituto Europeo di Oncologia, Milano, Italy

**Background:** Src-family kinases (SKFs) are involved in estrogen receptor (ER) and progesterone receptor (PgR) signaling pathways, in resistance to hormonal therapy, and in osteoclast function. Dasatinib is a potent oral inhibitor of SKFs and other kinases, but single-agent activity in advanced breast cancer (ABC) is limited. Biomarkers may identify patient subsets with increased benefit. In this Phase II study, the combination of dasatinib with exemestane was evaluated in patients (pts) with ER+ and/or PgR+ ABC resistant to a nonsteroidal aromatase inhibitor (NSAI).

**Methods:** In a randomized double-blind Phase II trial (CA180-261), 157 patients (pts) with ECOG performance status of 0-1, measurable or evaluable disease, and progression (PD) during, or within 1 year after adjuvant, treatment with an NSAI were stratified by symptomatic bone metastasis (SBM) and other factors, and assigned 1:1 to receive dasatinib (100 mg daily) or matched placebo in combination with exemestane (25 mg daily). Progression-free survival (PFS) was the primary endpoint and clinical benefit (CBR; partial response+stable disease ≥24 wks) the key secondary endpoint. The study was designed for 80% power to detect a hazard ratio (HR) of 0.67 between arms, corresponding to median PFS increase from 4 to 6 months using 1-sided α=0.1. Archival tumor samples for biomarker analysis were collected. Patient-reported pain score and a measure of bone lysis (urinary N-telopeptide, uNTX) were collected serially in pts with SBM.

**Results:** Planned analysis was performed after 119 events (PD or death) were recorded, at which time 129 pts had discontinued study treatment. Overall PFS comparison was non-significant (HR=0.86; 1-sided p=0.148), with median PFS of 16 weeks (95% CI 12, 18) in the placebo arm and 18 weeks (95% CI 15.24) in the dasatinib arm. Estimated free-from-progression rate at 24 weeks was 33% in placebo and 43% in the dasatinib arm. Pts with SBM, 40% of study population, had improved PFS on the dasatinib arm (HR=0.68, 1-sided p=0.094). Preliminary CBR at this analysis (31 of 38 censored pts continue on study) was higher in the dasatinib arm. Expected dasatinib-related toxicities were observed, including pleural effusion [23% (5% Gr≥3) in dasatinib vs 1% (0 Gr≥3) in placebo] and diarrhea [25% (1% Gr≥3) vs 1% (0 Gr≥3)]. Musculoskeletal adverse events (AEs) were comparable between arms, but fatigue or asthenia [37% (4% Gr≥3) vs 20% (0 Gr≥3)], skin AEs [28% (1% Gr≥3) vs 11% (0 Gr≥3)] and headache [22% (3% Gr≥3) vs 9% (0 Gr≥3)] were more common in dasatinib arm. Dose interruption or reduction was more frequent for dasatinib compared to placebo. Biomarker analyses are in progress.

**Conclusion:** PFS difference (HR=0.82) was not significant in overall study population, but higher CBR in the dasatinib arm and higher PFS in pts with SBM (HR=0.68) suggests that dasatinib has efficacy in a subset. The safety profile was consistent with dasatinib experience; AEs, including pleural effusion and diarrhea, were more common with dasatinib as compared with placebo. Updated efficacy and biomarker analyses will be presented.

**PD01-03**

**Src Is a Potential Therapeutic Target in Endocrine Resistant Breast Cancer Exhibiting Low Estrogen Receptor (ER)-Mediated Transactivation.**

Guest SK, Pancholi S, Patani N, Dowsett M, Johnston SR, Martin L-A. Institute of Cancer Research, London, United Kingdom; Royal Marsden Hospital, London, United Kingdom

**Aim:** Identification of the mechanisms governing sensitivity to dasatinib in endocrine resistant breast cancer.

**Background:** Despite the effectiveness of endocrine therapies over 40% of women relapse. To identify the molecular mechanisms associated with resistance to estrogen-deprivation on an aromatase inhibitor, we previously assessed changes in gene expression during adaptation to long-term culture of MCF7 human breast cancer (BC) cells in the absence of estradiol (E2) (LTED). Analyses of canonical signaling pathways highlighted FAK as one of the major pathways up regulated at the point of resistance and src as a dominant gene in this pathway. Src phosphorylates a plethora of proteins including ER. Surprisingly dasatinib a pan src inhibitor enhanced proliferation of MCF7 cells in the absence of E2 and had limited anti-proliferative effect in the LTED model. In contrast tamoxifen resistant MCF7 cells (TAMR) were exquisitely sensitive to dasatinib [1].

**Methods:** Proliferation was assessed using Titre Glo™. ER-mediated transcription was measured with an estrogen-response element linked luciferase reporter construct. Protein expression was determined by immunoblotting. Confocal microscopy was used to determine cellular localization of ER.

**Results:** Dasatinib decreased proliferation of the TAMR (IC50 0.05nM) and resensitized them to tamoxifen but had no effect on the wild-type (wt)-MCF7 or LTED cells. Previously a relationship between BC subtype and sensitivity to dasatinib was reported with basal and post-EMT BC cell lines showing the highest sensitivity, which associated with high caveolin (CAV) expression [2]. In ovarian cancer expression of CAV and urinokine plasminogen activator (uPA) also predicted sensitivity to dasatinib [3]. TAMR cells had a 2-fold increase in CAV-1 and 7-fold increase in uPA compared to wt cells. Comparison of LTED and TAMR showed that the TAMR whilst continuing to express high levels of ER, had a 10-fold lower level of ER-transactivation compared to wt-MCF7 and did not express PGR. In contrast the LTED showed a 7-fold increase in ER-transactivation compared to wt cells in the absence of E2. Strong evidence suggests ER in the TAMR cells acts via a non-genomic mechanism with ER expressed throughout the cell rather than being restricted to the nucleus. This implies the TAMR have an impaired luminal phenotype in relation to ER-genomic function. Dasatinib reduced the expression of ER in the TAMR and resulted in nuclear shuttling of ER whilst having no effect on the wt. BT474 (ER+/HER2 amplified) showed a similar distribution of ER to the TAMR cells but were far less sensitive to the antiproliferative effect of dasatinib. Of note the TAMR have increased phosphorylation of HER2 but no amplification.

**Conclusion:** These data suggest src has differential effects in these endocrine resistant cell lines. The anti-proliferative effect of dasatinib was more pronounced in the endocrine resistant cell line with low ER.
genomic activity. Src inhibition warrants further clinical evaluation as a therapeutic target in endocrine resistant breast cancer.

**PD01-04**
Entinostat, a Novel Histone Deacetylase Inhibitor, Added to Exemestane Improves PFS in Advanced Breast Cancer in a Randomized, Phase II, Double-Blind Study.
Yardley DA, Ismail-Khan RR, Klein PM, Sarah Cannon Research Institute, Nashville, TN; Tennessee Oncology, PLLC, Nashville, TN; H. Lee Moffitt Cancer Center, Tampa, FL; PMK Consulting, San Francisco, CA

**Background:** Entinostat is a novel, oral, class I-selective histone deacetylase inhibitor (HDACi) that has been shown pre-clinically to inhibit mechanisms of aromatase inhibitor (AI) resistance through epigenetically driven down-regulation of activated growth factor signaling pathways and normalization of estrogen receptor levels. This provided a strong rationale for the entinostat breast cancer (BC) program, which was designed to evaluate entinostat’s ability to increase and/or restore sensitivity to treatment with an AI. **Methods:** Postmenopausal women with ER+ advanced BC who had ≤ 1 prior chemotherapy and had progressed on a non-steroidal AI were randomized 1:1 to exemestane 25 mg daily + entinostat 5 mg or placebo weekly. Progression free survival (PFS) was the primary endpoint; objective response rate and clinical benefit rate were secondary endpoints and overall survival (OS) was an exploratory endpoint. Prospectively defined subsets of interest included patients with “AI-sensitive and AI-resistant disease”. AI-sensitive was defined as having had a PR, CR or SD for ≥ 6 months (mos) in the metastatic setting or having completed and remained disease free for ≥12 months post adjuvant therapy. All others were considered AI-resistant. Treatment continued until disease progression or unacceptable toxicity. Response assessments were performed every 8 weeks.

**Results:** 130 women were enrolled (64 exemestane+entinostat [E+E]; 66 exemestane+placebo [E+P]). All but 1 patient had Stage IV disease. 82% had measurable disease, of which 60% had visceral involvement. 42% had 1 prior line of hormonal therapy; 58% had >1. Analysis of the intent-to-treat (ITT) population showed significantly (defined prospectively as p<0.10) longer PFS with E+E than with E+P (median 3.72 vs. 1.78 mos; p<0.006) (CI 0.49, 1.09). While not powered, results for pre-defined subsets were consistent with improved PFS in the E+E arm. Interestingly, the subset of patients identified as AI-resistant, demonstrated a HR of 0.61 (CI 0.30, 1.25) (median 3.72 vs. 1.78 mos). While OS is still early, at 18 mos median follow-up at time of abstract submission, the E+E arm demonstrated a median OS of 26.94 mos compared to the E+P arm of 20.33 mos (HR = 0.56) (CI 0.31, 1.02). Entinostat combined with exemestane was well tolerated. The most frequent adverse events (AEs) were fatigue, gastrointestinal disturbances, and hematologic abnormalities. AEs with ≥ 20% higher incidence with E+E than E+P were fatigue (46% vs 26%) and uncomplicated neutropenia (25% vs 0%). Serious AE rate was similar for both arms (13% vs 12%).

**Conclusions:** Adding entinostat to exemestane resulted in improved PFS, the study’s primary endpoint. At a median follow-up of 18 mos, the exploratory endpoint of OS was also increased in the E+E arm. Importantly for a phase II study, the PFS benefit in all subsets evaluated was consistent with the overall improvement seen in the ITT. These data demonstrate that the addition of entinostat to AI therapy may prolong the duration of AI therapy, thereby delaying the initiation of subsequent therapies such as chemotherapy, a goal for many breast cancer patients. A phase III study is planned.

**PD01-05**
Histone Deacetylase Inhibitor LBH589 (Panobinostat) Suppresses the Activated-NFκB Pathway in Acquired Aromatase Inhibitor Resistant Breast Cancer Cells.
Kubo M, Kanaya N, Liu Z, Chen S. Beckman Research Institute of the City of Hope, Duarte, CA

**Background:** Aromatase inhibitors (AIs) are effective in treating postmenopausal patients with estrogen receptor (ER)-positive breast cancer. However, resistance to endocrine therapies remains a major problem in the management of ER-positive breast cancer. Recently, histone deacetylase inhibitors (HDACis) show promise as cancer therapeutics, but the full scope of their utility remains unknown. **Experimental Design:** In order to search for critical genes in developing resistance to AI, a microarray study was performed on AI resistant cells derived from MCF-7aro cells that overexpress aromatase and on AI resistant tumors from our mouse experiments. Moreover, using MCF-7aro cells that are resistant to anastrozole (Ana-R), letrozole (Let-R), and exemestane (Exe-R), as well as LTEDarO, we evaluated the significance of NFκB1 and several regulatory genes in AI resistance, and the anti-NFκB1 activity of HDACi LBH589 in vitro and in vivo.

**Results:** Significant changes (Fold change cutoff > 1.5, false discovery rate < 0.05) in the expression levels of 22 genes among three networks were identified from microarray analysis of our in vitro and in vivo models of acquired AI resistance. NFκB1 is a key player in one of three networks, including 11 genes, and is functionally up-regulated in all AI resistant cells. NFκB1 knockdown suppresses the proliferation of AI resistant cells more than that of MCF-7aro cells. Moreover, AI resistant cell lines are highly sensitive to LBH589 treatment and apoptosis is induced in these cell lines. LBH589 suppressed NFκB1 as demonstrated at both the mRNA and protein levels in LTEDarO and other AI resistant cell lines. Furthermore, the LBH589 treatment abrogated tumor growth in mice, and was associated with significant decreased levels of NFκB1 in tumors.

**Conclusions:** Our findings provide new insights into how the NFκB1-mediated networks play roles in AI resistance, and strongly support that LBH589 offers a novel therapeutic strategy for patients with AI resistant breast cancer.

**PD01-06**
Endoxifen Exhibits Potent Anti-Tumor Activity and Regulates Different Genes Than Tamoxifen in an Aromatase Expressing MCF7 Model Resistant to Letrozole.

**Background:** First in human studies of Z-endoxifen hydrochloride (E), the active metabolite of tamoxifen (T), are underway in metastatic breast cancer. **Experimental Design:** In order to search for critical genes in developing resistance to AI, a microarray study was performed on AI resistant cells derived from MCF-7aro cells that overexpress aromatase and on AI resistant tumors from our mouse experiments. Moreover, using MCF-7aro cells that are resistant to anastrozole (Ana-R), letrozole (Let-R), and exemestane (Exe-R), as well as LTEDarO, we evaluated the significance of NFκB1 and several regulatory genes in AI resistance, and the anti-NFκB1 activity of HDACi LBH589 in vitro and in vivo.

**Results:** Significant changes (Fold change cutoff > 1.5, false discovery rate < 0.05) in the expression levels of 22 genes among three networks were identified from microarray analysis of our in vitro and in vivo models of acquired AI resistance. NFκB1 is a key player in one of three networks, including 11 genes, and is functionally up-regulated in all AI resistant cells. NFκB1 knockdown suppresses the proliferation of AI resistant cells more than that of MCF-7aro cells. Moreover, AI resistant cell lines are highly sensitive to LBH589 treatment and apoptosis is induced in these cell lines. LBH589 suppressed NFκB1 as demonstrated at both the mRNA and protein levels in LTEDarO and other AI resistant cell lines. Furthermore, the LBH589 treatment abrogated tumor growth in mice, and was associated with significant decreased levels of NFκB1 in tumors.

**Conclusions:** Our findings provide new insights into how the NFκB1-mediated networks play roles in AI resistance, and strongly support that LBH589 offers a novel therapeutic strategy for patients with AI resistant breast cancer.
µg/day, sc), endoxifen 25 mg/kg/day p.o. (LDE) endoxifen 75 mg/kg/ day p.o. (HDE) or letrozole, 10 µg/day s.c. for 4 weeks. Tumors were harvested from control, T, and E groups while the L group continued treatment until the development of resistance defined as an increase in tumor volume of at least 300% from day 1. Mice with L-resistant tumors were randomly assigned to T (n=4) or E (n=5) for 4 weeks and then sacrificed. Gene expression in L-resistant tumors was quantified using Affymetrix U133+2 and changes in gene expression profiles [comparing T and E with L-resistant (n=3)] were analyzed. Genes identified as significantly different were confirmed by real-time RT-PCR assays.

Results: At the 4 week time point, both doses of E and L resulted in greater anti-tumor activity than control (Wilcoxon rank sum test: all p < 0.0001); however, tumor burden did not differ between T and control (p=0.095). HDE resulted in significantly less tumor burden than T (p=0.002) but was similar to L. In mice that continued on L, resistance developed at 24 weeks in 9/25 mice. These mice were randomly assigned to either T (n=4) or E (n=5) for 4 weeks. Tumor volume (expressed as a % of its size prior to randomization) was significantly different comparing E (73.3%; range: 69.3 to 80.75%) versus T (148.39%; range: 114.07 to 165.99%) (Wilcoxon rank sum test p=0.016). Compared to control, microarray studies identified 1518 unique probe sets regulated by E (p=0.001) compared to 441 for T including estrogen-regulated genes such as progestrone receptor (PGR) and amphiregulin (AREG) that were significantly down-regulated in the E group [PGR (-6.2 fold, p=0.000008) and AREG (-3.2 fold, p=0.0006) but unchanged or up-regulated in the T group (PGR unchanged and AREG +0.2 fold p=0.0002)]. These findings were confirmed by RT-PCR.

Conclusions: Using the MCF7/AC1 model previously used to show the superiority of AIs over T, HDE demonstrated similar anti-tumor activity to L and was superior to T. In cells resistant to L, E was superior to T and gene expression changes demonstrate that E down-regulates while T activates estrogen regulated genes. These findings support the ongoing development of E for the treatment of ER+ BC.

**PD01-07**

**AR Overexpression and Aromatase Inhibitor Resistance in Breast Cancer.**

Rechoum Y, Iacopetta D, Barone I, Ando 'S, Morales SF , Weigel NL, Fuqua SAW. Baylor College of Medicine, Houston, TX; University of Calabria, Calabria, Italy

Background: Aromatase inhibitors (AIs) have emerged as the therapy of choice for the treatment of estrogen receptor alpha (ERα) positive breast cancer. Although the involvement of the ERα in AI resistance is well established, the role of the androgen receptor (AR) is not known. It has been estimated that about 60%-70% of ERα-positive breast cancer co-express the AR, and that AR agonists can either inhibit or stimulate breast cancer cell proliferation. Thus it is important to determine if there are biomarkers predicting AR’s effects in breast tumors. We have previously shown a role for AR-overexpression in tamoxifen resistance in ERα-positive MCF-7 breast cancer cells; here we hypothesized that AR overexpression might similarly be involved in resistance to the AI anastrozole (Anas).

Materials and Methods: Stable transfection of MCF-7 cells was performed to generate cell lines that express the aromatase gene (MCF-7 BK Arom) and then co-transfected with an AR expression vector (MCF-7 AR Arom). Aromatase and AR expression levels were evaluated by western blot analysis, and the enzyme activity was evaluated using aromatase activity assays. Proliferation was tested using anchorage independent soft agar assays and MTT in the presence of the androgen substrate androstenedione (AD), or AD plus Anas. ERα and AR transcriptional activities were tested with ERE-luciferase reporter assays. Localization of ERα and AR within the cells was visualized using immunofluorescence microscopy. Results: ERα-positive MCF-7 cells were stably transfected with either aromatase, or aromatase plus AR. MCF-7 aromatase clones overexpressing AR were resistant to the growth inhibitory effects of Anas when stimulated with the androgen AD. Resistance was not mediated through changes in aromatase expression or activity. The growth of several of the AR Arom-overexpressing cells was stimulated with treatment of Anas alone, suggesting that Anas was acting as an agonist. As expected, AD treatment stimulated ERα transcriptional activity, but Anas was unable to block AD-stimulated activity in AR Arom-overexpressing cells using ERE-Luciferase reporter assay. Anas was able to enhance AR and ERα colocalization in AR-overexpressing cells. Resistance was not associated with activation of known mechanisms of resistance, such as HER2, IGF-1R, or MAPK. However AR-overexpressing cells had higher constitutive phosphorylation of FAK. Accordingly, resistance to Anas was blocked using an Akt1/2 inhibitor.

Conclusion: Using a model of ERα-positive breast cancer cells expressing exogenous aromatase and AR, we have demonstrated that AR overexpression confers resistance to the AI Anas. These results suggest that in patients recurring on hormonal therapy whose tumors express elevated levels of AR, targeted therapy to Akt might restore hormone sensitivity.

**PD01-08**

**Heterogeneity of Lapatinib Responses in HCC1954 HER2-Overexpressing Breast Cancer Cells Revealed by Single-Cell Automated Microscopy.**

Hardeman KN, Tyson D, Quaranta V. Vanderbilt University School of Medicine, Nashville, TN

In breast cancer, 20-30% of patients present with HER2 overexpression, making them potential candidates for targeted HER2 therapies such as lapatinib. Lapatinib is a dual, intracellular tyrosine kinase inhibitor of HER2 & EGFR and is standard care for metastatic breast cancer. Drug resistance to this type of therapy is a major cause of death in cancer patients. Intra-tumor heterogeneity with respect to drug response can be a driving force for relapse and/or drug resistance. In current drug response end-point assays (IC50), information on heterogeneity is lost because cell differences are averaged out over the whole population. To produce a more realistic appreciation of drug response variability within a tumor, our lab has developed an extension of high content automated microscopy, called Extended Time Resolved Automated Microscopy (ETRAM). ETRAM entails the visual tracking of single cells with fine temporal & spatial resolution, allowing for the calculation of proliferation, death, and quiescence rates from image stacks and movies. HER2+ breast cancer HCC1954 cells were fluorescently labeled w/ a nuclear tag (H2BmRFP) and treated w/ lapatinib in a dose-dependent manner, from 2000 nM to 0 nM (DMSO control). Using the ETRAM method we followed nearly 1000 single cells/treatment condition over 72 hours, acquiring images every 10-12 minutes. Unexpectedly, we discovered that small but significant subpopulations of HCC1954 cells were unaffected by high lapatinib concentrations (2000 nM) and continue to divide. Furthermore, a substantial subpopulation responds to high lapatinib by entering quiescence, not apoptosis. Since HCC1954 cells have a previously reported lapatinib IC50 of 300 nM, our data demonstrate a need to further investigate these subpopulations of cells in order to determine
their contribution to the IC50. Image stacks are being further analyzed to extract cellular metrics of division, quiescence and death rates in the presence of lapatinib. We are also investigating single cells by immunofluorescence of signaling molecules in the HER2 pathway, along with downstream targets such as PI3K, mTOR/AKT, SHC, and RAF1, to assess molecular differences regarding dividing and quiescent subpopulations in lapatinib, versus lapatinib-sensitive cells that apoptose. Our ETRAM-based single cell analyses may stimulate a re-interpretation of lapatinib responses in HER2-overexpressing breast cancer, and hopefully point to improved strategies of lapatinib treatment.

**PD01-09**


Jeff A, Ward TM, Iorns E, Gallas M, Aparicio SA, Pegram MD. Braman Family Breast Cancer Institute, Sylvester Comprehensive Cancer Center, University of Miami Miller School of Medicine, Miami, FL; UBC/BC Cancer Agency, Vancouver, Canada

Background: Overexpression of the epidermal growth factor receptor ERBB2 (HER2) is found in 20% of human breast cancers. Therapies targeting ERBB2 including trastuzumab and lapatinib have significantly improved the outlook for women with ERBB2+ breast cancer. However, resistance to these agents occurs frequently and remains a significant clinical problem. In the case of lapatinib resistance, the mechanism(s) of resistance remain poorly understood, since the current proposed rationale thought to limit lapatinib’s anti-tumor effects has been difficult to reconcile with clinical data. Therefore, we hypothesize that novel mechanisms of resistance could be identified by mapping genomic variations in ERBB2+ cells with acquired resistance to lapatinib. The identification of such mutations may provide insights into mechanisms of resistance and may indicate therapeutic strategies to overcome lapatinib resistance in ERBB2+ breast cancer.

Material and Methods: SKBR3 breast cancer cells resistant to lapatinib were generated through serial passage by exposure of drug sensitive parental SKBR3 cells to increasing concentrations of lapatinib up to the peak plasma concentration observed in human subjects (2.6 μM (SK-lapR)). Multiple signaling pathways in lapatinib sensitive and resistant cells were interrogated by Reverse Phase Protein Array (RPPA) and western blot analysis. To identify genome wide somatic mutations, the Exome of lapatinib resistant and sensitive SKBR3 cells was sequenced utilizing next generation deep sequencing. Following exclusion of germline variants, the acquired gene mutations in lapatinib resistant SKBR3 cells were confirmed by DNA re-sequencing of PCR amplified DNA segments.

Results and Discussion: Analysis of activated signaling pathways in lapatinib resistant and sensitive SKBR3 cells did not confirm any of the previously proposed mechanisms of resistance. In particular, these cells show no activation of AKT or alternative receptor tyrosine kinases such as IGF-IR, ERBB3 or c-Met. However they exhibit sustained activation of mTORC1 and ERK1/2, as well as phosphorylation of STAT3, STAT5, rpS6 and CREB. Initial sequence analysis of exome and transcriptome reveals the presence of 76 single nucleotide variants/Indels differing between sensitive and resistant cells with 34/76 validated as true mutations present in the genome of lapatinib resistant SKBR3 cells, including mutations in LAT52, MAP3K5, SMAD5 and PDGFRA. This is the first exome sequence analysis to be reported which defines a drug resistant phenotype in ERBB2+ breast cancer. Ongoing work includes investigation of mutations as drug resistance mediators and analysis of copy number variations and gene fusions/translocations to systematically search for molecular alterations, with the goal of providing a rationale for the design of new combination therapies aimed at lapatinib resistance for ERBB2+ breast cancer.

**PD02-01**

Impact of Contralateral Prophylactic Mastectomy on Surgical Outcomes.


Background: Among women with unilateral cancer, rates of contralateral prophylactic mastectomy (CPM) are continuing to increase. However, little is known about whether rates and types of complications differ between patients undergoing unilateral mastectomy or bilateral mastectomy, limiting the surgical outcomes evidence that can be presented in pre-surgical decision making for women considering CPM. This study was undertaken to determine whether surgical complications are increased in women undergoing CPM compared to those without CPM.

Methods: Between the years 2005-2010, all patients at UCSF undergoing mastectomy with immediate reconstruction were entered into a prospective database. This database was queried for patients with unilateral cancer who had mastectomy and immediate reconstruction with or without CPM. Surgical outcomes, including implant loss, admission for IV antibiotics, and return to OR were evaluated and compared between patients who did and did not undergo CPM. Patients with bilateral cancer or bilateral prophylactic surgery were excluded; analyses were limited to patients with a minimum of 1 year follow-up.

Results: 468 patients were identified who met study criteria, totaling 667 breasts. Mean follow-up was 22 months (range 12 – 69 months). 269 of the 468 (57.5%) patients had unilateral mastectomy only, while 199 of 468 (42.5%) patients also had CPM. There were no differences in tumor grade, stage, follow-up time, smoking history, or radiation (prior or post-surgery) between the two groups. The only significant differences between the unilateral and bilateral groups were median age at diagnosis (50.7 vs. 45.9 respectively; p < .0001) and receipt of neoadjuvant chemotherapy (34.7% vs. 41.3% respectively; p < .01). Surgical outcomes were compared between groups. The overall rate of major complications differed significantly due to an increased rate of infectious complications and unplanned return to surgery in the CPM group (Table 1). Nevertheless, this did not result in a higher implant loss rate in the CPM group.

<table>
<thead>
<tr>
<th>Type of Surgery</th>
<th>Major Complication1</th>
<th>Implant Loss2</th>
<th>IV antibiotics3</th>
<th>Unplanned Return to OR4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unilateral</td>
<td>72/269 (26.77%)</td>
<td>50/269 (18.52%)</td>
<td>54/269 (20.12%)</td>
<td>63/269 (23.42%)</td>
</tr>
<tr>
<td>Bilateral</td>
<td>81/199 (40.79%)</td>
<td>51/199 (25.58%)</td>
<td>63/199 (31.61%)</td>
<td>63/199 (31.61%)</td>
</tr>
<tr>
<td>All patients</td>
<td>153/468 (32.69%)</td>
<td>101/468 (21.65%)</td>
<td>117/468 (25.14%)</td>
<td>126/468 (27.13%)</td>
</tr>
</tbody>
</table>

p < .01; 1'st not significant

In patients undergoing bilateral mastectomy, overall complication rates were comparable between the index breast and the CPM breast; however, there was a higher implant loss rate in the index breast (22/177 vs. 11/188; p=0.05).

Conclusions: While CPM is an increasingly common procedure, it is associated with an increased risk of major post-operative surgical complications. In this cohort, patients undergoing bilateral mastectomy for unilateral cancer had higher rates of overall complications, greater use of IV antibiotics, and more frequent return to the operating room. Since the majority of CPM cases are not at sufficiently high risk for a second breast cancer to meet clinical criteria
for prophylactic surgery, guidelines and clinical recommendations should consider these increased complication rates when counseling women contemplating CPM.

PD02-02
A Decision Analysis of Contralateral Prophylactic Mastectomy in Women Undergoing Treatment for Sporadic Unilateral Breast Cancer.
Lester-Coll NH, Lee JM, Gogineni K, Hwang W-T, Schwartz JS, Prosnitz RG. University of Pennsylvania Perelman School of Medicine, Philadelphia, PA; Massachusetts General Hospital, Boston, MA; Abramson Cancer Center, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA

Background:
The intent of contralateral prophylactic mastectomy (CPM) is to improve survival after a diagnosis of unilateral breast cancer by reducing the risk of contralateral breast cancer (CBC). CPM rates are rising among women with sporadic breast cancer, despite limited evidence that its benefits outweigh its harms. Although CPM is highly effective in reducing a woman’s risk of CBC, the competing mortality risk from a patient’s index breast cancer may offset its benefits. Furthermore, any examination of CPM needs to consider quality of life effects.

Methods:
We developed a Markov decision analytic model to estimate the effect of CPM in women with newly diagnosed unilateral breast cancer. The primary outcomes examined were gains in life expectancy (LE) and quality-adjusted life expectancy (QALE) for CPM compared with no CPM in 18 hypothetical cohorts of 45-year-old women. Data from the British Columbia Cancer Agency (BCCA) was used to generate AJCC stage and molecular subtype-specific estimates of the risk of developing distant metastases from an index breast cancer. A correction factor was applied to account for the omission of relevant systemic therapy (including trastuzamab) in some women in the BCCA cohort. Additional model parameters, including utilities (quality of life weights) for breast cancer and CPM health states, were identified from the published medical literature. LE and QALE estimates were not discounted in the base case. Univariate sensitivity analysis was used to examine the impact of plausible variation in the key model parameters on model results.

Results:
CPM improved LE in all cohorts (range: 0.06 - 0.54 years, Table 1). AJCC stage had more effect on LE than molecular subtype (stage I mean, 0.43 years, stage III mean, 0.11 years). However, after adjusting for quality of life, a strategy of no CPM was favored in all cohorts. Univariate sensitivity analysis demonstrated that the only model parameter that influenced the outcome of QALE was the utility for health after CPM. In the base case the utility after CPM was 0.81 (compared to 0.85 for No CPM). The preferred strategy did not change from No CPM to CPM unless the utility after CPM exceeded 0.83. Model results were otherwise stable across the ranges of the key parameters examined, including the risk of distant metastases resulting from a patient’s index breast cancer by stage and subtype, duration of survival with metastatic breast cancer, and the risk of CBC.

Conclusions:
The primary drivers of survival after unilateral breast cancer are stage at diagnosis and molecular subtype. Our model demonstrates that CPM confers modest additional LE gains, even in women with early-stage, favorable-subtype breast cancer. Furthermore, this modest benefit is negated if one assumes a small reduction in quality of life due to CPM. The decision to pursue CPM as part of treatment of unilateral breast cancer should include consideration of both patient specific breast cancer characteristics and individual preferences.

<table>
<thead>
<tr>
<th>Molecular Subtype</th>
<th>AJCC I</th>
<th>AJCC II</th>
<th>AJCC III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luminal A</td>
<td>0.49</td>
<td>0.38</td>
<td>0.18</td>
</tr>
<tr>
<td>Luminal B</td>
<td>0.37</td>
<td>0.22</td>
<td>0.09</td>
</tr>
<tr>
<td>HER2</td>
<td>0.54</td>
<td>0.35</td>
<td>0.14</td>
</tr>
<tr>
<td>Luminal/HER2</td>
<td>0.42</td>
<td>0.26</td>
<td>0.14</td>
</tr>
<tr>
<td>Basal-like</td>
<td>0.17</td>
<td>0.14</td>
<td>0.04</td>
</tr>
<tr>
<td>Triplike-Non-Basal</td>
<td>0.41</td>
<td>0.20</td>
<td>0.06</td>
</tr>
</tbody>
</table>

PD02-03
The Effect of Breast Conservation Therapy vs Mastectomy on Symptoms, Physical Impairments, and Function.
Kesarwala AH, Pfalzer LA, O’Meara WP, Stout NL. National Cancer Institute, Bethesda, MD; University of Michigan - Flint, Flint, MI; National Naval Medical Center, Bethesda, MD

Background: Early-stage breast cancer (BC) patients choose between breast conservation therapy (BCT) and mastectomy based on comparable recurrence rates and overall survival. In the absence of mortality benefit, consideration of anticipated functional impairments could guide decision making. Although BCT offers less extensive surgery, the administration of radiation therapy (RT) may adversely impact upper extremity (UE) function. The purpose of this analysis is to investigate the effect of BCT vs modified radical mastectomy (MRM) without RT on functional impairments among BC survivors.

Materials and Methods: 196 women diagnosed with BC between 2001-05 were enrolled and treated in a prospective surveillance physical therapy program. 115 received either BCT, including lumpectomy and whole breast RT, or MRM without RT and were analyzed for this report. Participants’ UE range of motion (ROM), strength, and limb volume were assessed pre-operatively and at 1, 3, 6, 9, and 12+ months post-operatively by a physical therapist. Limb volume was assessed using infrared optoelectronic perometry. At 12+ months, overall health status, UE symptoms and function, and physical activity levels were reported using standardized questionnaires. Analysis of variance estimated differences in impairments and self-reported symptoms and function. One-way ANOVA analysis was used to determine significance between groups (p ≤ 0.05).

Results: 65 women (57.5%) received BCT and 50 women (42.5%) received MRM. No significant differences in age, BMI, stage, ER/PR status, and number of dissected lymph nodes were found between groups. At 1 month post-operatively, shoulder internal rotation (p = 0.03), abduction (p = 0.01), and flexion (p = 0.004) were worse in post-MRM patients, with a trend towards worse external rotation (p = 0.06). A higher rate of axillary cording was seen in patients post-MRM (p = 0.02). By 12+ months post-operatively, there were no differences in any of the shoulder ROM variables. BCT patients reported, however, greater weakness (p = 0.03) and diminished ability to perform heavy household tasks (p = 0.03). There was no significant difference between BCT vs. MRM in rates of early lymphedema (40% vs 38%) or seroma (14% vs 22%).

Conclusion: Functional impairments represent an important category of morbidity for BC survivors and should be considered in pre-treatment decision making. Post-operative RT as part of BCT may not contribute significantly to impairment over the first year of treatment. The presence of self-reported weakness and difficulty performing heavy household tasks at 12+ months suggest possible future functional deficits, especially considering the potentially progressive nature of RT-associated tissue changes. Additional research is needed to assess longer-term changes and the impact of RT in the context of aggregate effects of other BC treatment modalities.
PD02-04
A Randomized, Prospective, Multicenter Study of the Impact of Intraoperative Margin Assessment with Adjunctive Use of MarginProbe vs. Standard of Care. 
Schnabel F, Trafta L, The MarginProbe Study Group. NYU Langone Medical Center, New York, NY; The AAMC Breast Center, Annapolis, MD; Dune Medical Devices

Background: The current practice of breast conserving surgery (BCS) involves intraoperative margin assessment according to the surgeon’s gross assessment and judgment. The failure of this intraoperative assessment has been associated with a 20-30% reoperation rate to assure negative margins. MarginProbe (Dune Medical Devices) was developed to provide real-time assessment of lumpectomy specimens to evaluate for the presence of disease at the surgical margins. A 21-center international pivotal study was conducted to determine if adjunctive use of MarginProbe can enable surgeons to identify positive margins intraoperatively, resulting in fewer patients who are candidates for re-excision procedures.

Methods: 664 women with non-palpable lesions undergoing lumpectomy for DCIS and invasive cancer were enrolled and 596 randomized (1:1) in the operating room following standard of care (SOC) lumpectomy. In the device arm, MarginProbe was used to assess all surfaces of the lumpectomy specimen and positive readings required additional resections. All specimens were inked in the operating room by the surgeon. All specimens were examined to verify excision of the target lesion intraoperatively. Pathologists were blinded to study arm. Additional surgeries to re-excite involved margins were performed per each individual site criteria. Patients were followed for 2 months following surgery; additional procedures were documented. Safety was assessed by reports of adverse events.

Results: No safety concerns were raised. Of the 298 patients in each arm, 55% (163/298) patients in the device arm (D) and 49% (147/298) in the control arm (C) had at least one positive margin on the main lumpectomy specimen (≤1mm). Surgeons’ ability to identify and resect all positive margins per patient was significantly improved with device use (D: 72% (117/163), C: 22% (33/147); p < 0.0001). Following lumpectomy, the number of patients having positive margins due to failed intraoperative assessment (excluding skin or fascia) was reduced by 57% in the device arm (D: 42/298(14%), C: 98/298(32.8%), p=0.0001), leading to a 50% reduction in re-excisions for this group (D: 31/298 (10.4%), C: 62/298 (20.8%); p=0.001). There was no significant difference in tissue volume removed (D: 93cc, C: 85cc; 25cc non-inferiority delta, p=0.0001). Normalized to baseline breast size, tissue volume in the device arm was non-inferior to the control arm (D: 15.1%, C: 12.5%, 5% non-inferiority delta, p <0.0001). For patients having at least one positive margin prior to intraoperative assessment, there was a trend in the device arm towards smaller average tissue volume resected to achieve clear margins (D: 99cc, C: 108cc; p=0.6).

Conclusions: Adjunctive use of MarginProbe during BCS significantly improved surgical outcomes by improving surgeons’ ability to identify and immediately resect positive margins, reducing the number of patients requiring re-excision. Overall tissue volume removed was not statistically different in the two arms. Patients in the device arm who had positive margins prior to intraoperative assessment had less average tissue volume removed and required fewer re-excisions to achieve appropriate margins.

PD02-05
MRI Phenotype and Tumor Subtype Affect Breast Conservation Eligibility and MRI Accuracy in the I-SPY 1 Trial.
Mukhtar RA, Hylton N, Rosen M, The I-SPY 1 Trial Investigators, ACRIN 6657 Investigators, Esserman LJ. UCSF; University of Pennsylvania; I-SPY 1 Trial; ACRIN 6657

Background
Neoadjuvant chemotherapy (NAC) in breast cancer allows assessment of response to treatment and increases eligibility for breast conservation treatment (BCT). We previously reported that MRI tumor phenotype (based on loss of containment) predicted response to NAC and ability to achieve BCT. Since tumor subtype can affect MRI features, we sought to validate whether MRI phenotype and/or tumor subtype affected BCT eligibility in a prospective trial, I-SPY (CALGB 150007/ACRIN 6657). We also investigated the accuracy of post-NAC MRI in determining BCT eligibility, and the distribution of tumor subtypes within MRI phenotypes with the goal of identifying clinically useful factors associated with successful BCT.

Methods
We analyzed I-SPY 1 Trial data, a multi-center, prospective NAC trial with serial MRIs. Phenotypes 1 and 2 were well defined masses, while 3, 4, and 5 were more diffuse. Subjects were considered truly eligible for BCT if tumor diameter on surgical pathology was ≤ 4 cm (based on NSABP B-06 trial criteria). Post-NAC MRI and surgical pathology were considered discrepant if longest tumor diameter differed by > 2 cm. Categorical variables were compared using the chi-squared test.

Results
Of the 221 subjects, 198 had pre and post-NAC MRIs, and surgical pathology available. Of these, 174 had a tumor diameter ≥ 4 cm prior to NAC, and were considered initially ineligible for BCT. After NAC, 141 (81%) patients became BCT eligible based on having ≤4 cm of residual tumor. In contrast, 46 (26%) had pathologic complete response (pCR, no invasive tumor). The rate of becoming BCT eligible differed by hormone receptor (HR)/Her2 status (p = 0.005) and MRI phenotype (p = 0.037), with higher rates of eligibility in Her2+ and triple negative tumors, and the well defined MRI phenotypes. Overall, 75 (38%) patients had a size discrepancy between tumor diameter on post-NAC MRI and size on surgical pathology. Subjects with diffuse MRI phenotypes had more size discrepancy than phenotypes 1 and 2 (p = 0.001). Subjects with HR+/Her2- tumors also had more size discrepancy than other subtypes (p = 0.004). The more diffuse MRI phenotypes were more commonly HR+/Her2- tumors, and less commonly HR+/Her2- (p = 0.006). There was no difference in actual receipt of BCT by HR/Her2 status. However, patients with a well-defined MRI phenotype were significantly more likely to actually receive BCT than those with a diffuse MRI phenotype (47% versus 27%, p = 0.023).

Conclusions
Patients with well defined patterns on MRI and with Her2+ or triple negative disease were more likely to become eligible for BCT after NAC. The same factors that predict pCR also predict BCT eligibility, but far more patients achieve BCT eligibility than pCR. MRI after NAC was less accurate in the diffuse phenotypes, which could reflect increased difficulty in measuring tumor size in this setting. Tumor phenotype and subtype can help predict likelihood of achieving eligibility for BCT, and can be useful in setting appropriate expectations for women at the start of NAC.
Outcomes after Total-Skin Sparing Mastectomy and Immediate Reconstruction in 657 Breasts.

Warren Peled A, Stover AC, Foster RD, Alvarado MD, Ewing CA, Hwang ES, Esserman LJ. University of California, San Francisco

INTRODUCTION: Total skin-sparing mastectomy (TSSM) is increasingly offered to women for both therapeutic and prophylactic indications. When combined with immediate breast reconstruction, patients can achieve excellent aesthetic results and high satisfaction. However, the oncologic safety of the procedure remains controversial. Further, the technique can be associated with higher rates of post-operative ischemic complications. We conducted this study to determine oncologic and ischemic outcomes in a large cohort of patients undergoing TSSM and immediate reconstruction.

METHODS: Patient and tumor characteristics and treatment details were collected in a prospectively maintained database. All patients undergoing TSSM and immediate breast reconstruction at our institution from 2001 to 2010 were included in the analysis. The development of any post-operative complications or local or distant recurrence was determined.

RESULTS: TSSM with immediate reconstruction was performed in 428 patients for a total of 657 breasts. Mean patient age was 46.9 years. 210 patients (49%) had neoadjuvant chemotherapy for locally advanced disease. 114 patients (26.7%) had post-mastectomy radiation therapy. 54% of patients had bilateral mastectomies. Prophylactic mastectomies (either unilateral or bilateral) accounted for 244 (37.1%) of cases, which included bilateral mastectomies in 15 patients (30 cases) who were known BRCA-1 or -2 mutation carriers. Expander-implant reconstruction was performed in 80% of the cases, while the rest of the cases involved autologous reconstruction (15.3%) or immediate implant placement (4.7%). On pathologic examination, nipple tissue from 11 breasts (1.7%) contained in situ cancer and from 9 breasts (1.4%) contained invasive cancer; re-excision was performed in 7 of these cases, the nipple-areolar complex was removed entirely in 9 cases, and radiation therapy was given without further excision in the rest of cases. Ischemic or necrotic post-operative complications included 13 cases (1.9%) of partial nipple loss, 10 cases (1.5%) of complete nipple loss, and 78 cases (11.8%) of skin flap necrosis or incisional dehiscence. At a median follow-up of 23 months (range 3-116 months), 5 patients (1.2%) had developed a local recurrence alone, 10 patients (2.4%) had developed a distant recurrence alone, and 6 patients (1.4%) had developed both loco-regional recurrence and distant metastases. In the subset of patients with at least 3 years’ follow-up, rates of local and of distant recurrence were 1.7% at a median of 45 months follow-up. None of the patients who underwent bilateral prophylactic mastectomy for BRCA-1 or -2 mutations developed subsequent breast cancers.

CONCLUSIONS: In this large, high-risk cohort, TSSM was associated with low rates of nipple involvement and loco-regional recurrence. Ischemic complications, although uncommon, often resulted in nipple loss. These short-term outcomes are encouraging, although longer follow-up will be important for confirmation of long-term oncologic safety. Serial improvements in surgical technique can improve selection criteria and reduce post-operative complication rates.

Models Predicting Non-Sentinel Node Involvement in Breast Cancer Also Predict for Regional Recurrence If the Axilla Is Not Treated.

Pepels M, Vestjens H, de Boer M, Bult P, van Dijck J, Mencke M, van Diest P, Borm G, Tjan Y. Maastricht University Medical Centre; Radboud University Nijmegen Medical Centre; Erasmus Medical Center Rotterdam; University Medical Centre Utrecht

Background

Series on breast cancer patients with sentinel node (SN) isolated tumor cells or micrometastases show low recurrence rates in the presence of SN isolated tumor cells, and varying recurrence rates in the presence of SN micrometastases. Non-SN prediction models are frequently used as a decision aid to identify patients that may not need axillary treatment, but this still needs to be validated in respect to regional recurrence.

Patients and Methods

We followed a cohort of 486 patients in the MIRROR-study of whom none received axillary treatment with favorable primary tumor characteristics and pN0(i+)(sn) or pN1(mi)(sn) for a median of 5 years. The patients were categorized into low or high-risk subgroups based on several published non-SN prediction models (cut-off levels between models varied from 3 to 10%), i.e. the Tenon Scoring system, MSKCC nomogram, Stanford nomogram and a Dutch model (Bolster et al.). The primary endpoint was 5-year regional recurrence-free survival.

Results

The overall 5-year regional recurrence rate was 3.0% (+/- 0.1%). Using the Tenon scoring system, we identified 438 patients with a low risk score of 3.5 or lower with a 5-year regional recurrence rate of 2.3% (+/- 0.8%), compared to a recurrence rate of 10.1% (+/- 0.4%) in 48 patients with a score above 3.5. For the MSKCC nomogram a low risk score of 0.10 or lower identified 300 patients with a 5-year recurrence rate of 2.8% (+/- 1.1%), and a score above 0.10 identified 166 patients with a recurrence rate of 3.4% (+/- 1.5%) (20 patients not assessable). By the Stanford nomogram a low risk cutoff level of 10% identified 21 patients without recurrence, whereas 465 patients had a 3.2% (+/-0.9%) recurrence rate. Using a Dutch model, a low risk cutoff score of 20 discriminated between 384 patients with a 5-year recurrence rate of 2.2% (+/- 0.8%) and 102 patients with a recurrence rate of 6.3% (+/- 2.9%). Further analyses with different cut-off values and subgroups will be presented at the conference.

Conclusion

Using several published non-SN prediction models for predicting regional recurrence, the Tenon scoring system outperformed the other models in that it identified the largest subgroup of patients with a low recurrence rate. We would recommend axillary treatment in patients classified as high risk according to the Tenon score.

Funding: Netherlands Organization for Health Research and Development (ZonMW 945-06-509).

Validation over Time of a Nomogram Predicting the Sentinel Node Positivity in Early Breast Carcinoma According to the Molecular Subtypes Classification.


Background: The molecular subtypes of breast cancer have different axillary status. A new nomogram including the interaction covariate between estrogen receptor (ER) and HER2 status has been recently
published (Reyal et al. PLOSone, May 2011) and allows to identify before surgery the patients with a high risk of positive sentinel lymph node (SLN). The purpose of our study was to validate this model on an independent population.

**Patients and methods:** We studied 755 consecutive patients treated for operable breast cancer with sentinel node biopsies in 2009, from the Institut Curie breast cancer prospective database. Baseline characteristics were compared between our population and the population used to build the model, using Chi-square test for categorical variables and Kruskal-Wallis test for continuous variables. The multivariate model, including age, tumor size, lymphovascular invasion and interaction covariate between ER and HER2 status, was used to calculate the theoretical risk of positive sentinel lymph node (SLN) for all patients. The performance of the model on our population was then evaluated in terms of discrimination (area under the curve AUC) and of calibration (Hosmer-Lemeshow HL test).

**Results:** Characteristics of our population were significantly different from the training population for the following variables: tumor size (median 12mm [1-60] versus 13mm [1-100] p=0.005), lymphovascular invasion (18.6% versus 23.7% p=0.006), positive ER (91.4% versus 87% p=0.002) and age as followed: 56.7% of patients ≤ 60 versus 63.1%, 17.5% of patients between 60 and 65 versus 14.1% and 25.8% of patients above 65 versus 22.8% p=0.01. The nomogram showed similar results in our population than in the training population in terms of discrimination (AUC= 0.72 [0.68-0.76] versus 0.73 [0.7-0.75]) and calibration (HL p= 0.4 versus p=0.35).

**Conclusions:** Despite significant differences between the two populations concerning variables which are part of the nomogram, the model was validated in our population. Our study shows that this nomogram is efficient and robust over time to predict the likelihood of positive SLN according to molecular subtypes defined by surrogate markers ER and HER2 determined by immunohistochemistry in clinical practice.

**PD02-09**
Withdrawn by Author

**PD03-01**
**An Integrated Analysis of Three Distinct IBC/nIBC Affymetrix Gene Expression Data Sets Further Unveils the Molecular Biology of IBC.**

Van Laere SJ, Ueno NT, Finetti P, Vermeulen PB,ucci A, Birnbaum D, Robertson FM, van Dam PA, Woodward WA, Viens P, Dirix LY, Reuben JM, Iwamoto T, Cristofanilli M, Bertucci F; Oncology Center - GH Sint-Augustinus, Wilrijk, Antwerp, Belgium; The University of Texas MD Anderson Cancer Center, Houston, TX; Institut Paoli-Calmettes (IPC), Marseille, France; World IBC Consortium; Contributed Equally

Introduction. Several studies have applied gene expression profiling to inflammatory breast cancer (IBC). Most of these studies were underpowered. Here, we present an integrated analysis of 3 distinct gene expression data sets of IBC and non-IBC (nIBC) samples to further uncover the IBC-specific molecular biology with enhanced statistical power.

Materials & Methods. Three Affymetrix gene expression data sets were combined, resulting in a series of 137 IBC and 252 nIBC samples. IBC was diagnosed clinically. Each sample was classified according to several published gene signatures. Transcriptional heterogeneity was investigated using hierarchical clustering, coupled with silhouette score analysis. IBC-specific, molecular subtype-independent differences in gene expression were identified using linear regression modeling. Differentially expressed genes were translated into pathways using Ingenuity Pathway Analysis. Cox regression analysis was used to identify variables influencing distant metastasis-free survival (DMFS) in IBC. Finally, we focussed on the molecular aspects of pathological response to neoadjuvant chemotherapy in patients with IBC.

Results. In our series of IBC samples, 4 robust sample clusters were identified. These sample clusters were mainly associated with the different molecular subtypes (P=0.0001), all of which were identified in IBC with a similar prevalence in nIBC, except for the Luminal A subtype (9% vs. 40%; P=0.0001) and the ErbB2+ subtype (23% vs. 8%; P=0.0002). A total of 632 genes were differentially expressed. Analysis of this gene list identified an IBC-repressed network centered on TGFβ. Activated TGFβ-profiles and SMAD-profiles in the nIBC samples corroborated these findings. Consistent with published poor prognosis signatures, current survival analysis indicated that the molecular subtypes are significantly associated with prognosis in IBC. Surprisingly, in IBC, the Luminal A samples exhibited the shortest DMFS-interval (HR=4.02; P<0.05). Comparison of responders and non-responders to neoadjuvant chemotherapy suggests a prominent role for inflammation/immunity-related processes in determining the efficacy of neoadjuvant chemotherapy in IBC.

Conclusions. IBC, like nIBC, is transcriptionally heterogeneous as exemplified by the identification of 4 robust sample clusters in the present series. This observation is further corroborated by the identification of all known molecular subtypes in IBC, albeit with a different distribution pattern characterized by a low frequency of Luminal A samples. Nevertheless, this phenotype is clinically relevant, as demonstrated by the poor prognosis profile. Our observations can be explained by the IBC-specific repression of TGFβ, which is a key molecule of epithelial-to-mesenchymal transition and is also known to prevent ER-expressing cells from proliferating. Finally, as in nIBC, inflammation- and immunity-related processes are important aspects of response to neoadjuvant chemotherapy in IBC.

**PD03-02**
**Prognostic and Predictive Predictors for Triple Negative Breast Cancer.**

Karn T, PusztaI L, Ruckhäberle E, Liedtke C, Schmidt M, Müller V, Gütte B, Hanker L, Ahr A, Holtrich U, Rody A, Kaufmann M; Goethe University, Frankfurt, Hessen, Germany; University of Texas M.D. Anderson Cancer Center, Houston, TX; University of Münster; University of Mainz; University Hospital Hamburg-Eppendorf; Saarland-University

Background: Both the prognosis and the therapeutic options in triple negative breast cancer (TNBC) are rather limited. Current prognostic gene expression profiles for breast cancer mainly reflect proliferation status and are most useful in ER-positive cancers. The identification of prognostic gene signatures from TNBC cohorts in previous studies was hindered due to relatively small sample sizes.

Materials and Methods: All currently available TNBC gene expression datasets generated on Affymetrix U133 gene chips were assembled. To minimize inter-laboratory variation we analyzed only highly comparable arrays and data set-biased genes were filtered. Supervised analysis was applied to identify a prognostic signature from a finding cohort of 394 TNBC and validation was performed in an independent cohort of 261 TNBC. The genes from the prognostic predictor were analyzed for their correlation to known molecular phenotypes among TNBC.

Results: Two supervised prognostic signatures consisting of 264 and 26 probesets, respectively, were obtained when applying
different cutoffs for false discovery rates of 25% and < 3.5% in the finding cohort. In multivariate analysis in the independent validation cohort hazard ratios of 4.03 (95% CI 1.71-9.48; P=0.001) and 4.08 (95% CI 1.79-9.28; P=0.001), respectively, were obtained for the two signatures. When compared to 16 metagenes for previously described molecular phenotypes in TNBC the prognostic signatures displayed highest correlation to metagenes for IL-8/ inflammation, VEGF/angiogenesis, and Histones. A subset of genes in the 264-probeset signature was inversely associated with a poor prognosis (29/264=11.0%). Most of these “good prognosis” genes are correlated with immune cell metagenes (21/29=72.4%). In contrast both identified supervised prognostic signatures did not correlate to previously published prognostic signatures (recurrence score, genomic grade index, Amsterdam signature, wound response signature, 7-gene immune response module, stroma derived prognostic predictor, and a medullary like signature). Regarding the response of TNBC to neoadjuvant chemotherapy the predictive value of the B-cell metagene was superior to the 264- and 26-probeset signatures. However combination of the B-cell metagene and the signatures increased the AUC in ROC-analysis from 0.606 to 0.656.

Conclusions: The use datasets consisting only of TNBC allows identification supervised prognostic signatures for TNBC which are unrelated to previously known prognostic signatures.

**PD03-03**
Identification of Transcription Factors Critical for the Growth of Basal Breast Cancer.
Shepherd JH, Mazumdar A, Tsimelzon A, Hilsenbeck SG, Brown PH. Baylor College of Medicine, Houston, TX; The University of Texas MD Anderson Cancer Center, Houston, TX

**Background:** Basal breast cancers are aggressive, poor prognosis tumors that occur commonly in young women and in African American women. Profiling of breast tumor mRNA has demonstrated that there are differences in gene expression between the basal and luminal subtypes of breast cancer. In this study, we identified response elements in the genes that define basal breast cancers, and identified transcription factors that are critical for the growth of basal breast cancer cells.

**Materials and Methods:** We performed promoter analysis using 4 published microarray studies (PMID: 12829800; PMID: 19435916; PMID: 17157792; PMID: 11562467) to select genes that are highly expressed in basal tumors compared to luminal tumors. For this analysis we selected 61 genes highly expressed in a set of basal breast tumors in any of the 4 microarray studies. We next used the online tool, CORE TF, along with the MATCH algorithm minimizing for the sum of false positives and false negatives to identify binding motifs within the promoter (defined from -1 kb to the first exon) of each gene. The frequency of binding motif occurrence for these 61 basal genes was compared to the frequency within the promoters of 3000 randomly selected genes. Significance was tested using an exact binomial test with a cutoff of p=0.05. RNA expression of motif-identified transcription factors was then analyzed in-silico using all 10 datasets in Oncomine™ that contained annotation for triple-negative status. Expression in triple-negative samples was compared to expression in non-triple-negative samples with a cutoff of p=0.05. We next performed siRNA knockdown studies to determine whether the identified TFs regulate basal breast cancer growth. Basal and luminal cells transfected with control and specific siRNAs were grown in triplicate and mean cell counts at day 6 were compared.

**Results:** Promoter analysis identified 24 binding motifs that were over-represented in basal breast tumor genes compared to a random set of 3000 genes. TransFac analysis indicated that 47 transcription factors bind the 24 identified motifs. Oncomine analysis showed that 8 of the 47 transcription factors were significantly more highly expressed in basal as compared to non-basal tumors. Identified transcription factors include FOXC1, FOXM1, CDC5L, E2F3, CEBP and NF-Y. siRNA to FOXM1 in 2 basal breast cell lines reduced growth by >70% after 6 days, whereas, in the luminal cell line MCF7, growth was reduced by 15%.

**Discussion:** This study identified transcription factors that are highly expressed in basal breast tumors (as compared to non-basal breast tumors). siRNA knockdown studies showed that FOXM1 is critical for basal breast cancer cell growth. These results suggest that transcription factors highly expressed in basal breast cancers may be novel targets for the treatment of this disease.

These studies were supported by a Promise grant from the Susan G. Komen for the Cure (PB, SGH), and by the Norman E. Brinker Award for Research Excellence (PB).

**PD03-04**
SuperPathway Analyses of Luminal and Basaloid Breast Cancers from the Cancer Genome Atlas (TCGA) Program.
Yau C, Benz S, Sanborn JZ, Stuart J, Haussler D, Benz C. Buck Institute for Research on Aging, Novato, CA; UC Santa Cruz, Santa Cruz, CA

The biological and clinical heterogeneity of breast cancer is clearly evident by its different intrinsic transcriptional subtypes. With exception of the HER2 subtype, pathways and signaling networks driving and distinguishing the other major breast cancer subtypes (basaloid, luminal-A, luminal-B) remain largely undefined. As of April 2011, >300 TCGA breast cancer samples have been characterized by both genomic copy number analysis and mRNA expression profiling (44% luminal-A, 26% luminal-B, 16% basaloid). As well, overall survival (OS) data are presently available on 104 luminal-A and 52 luminal-B cases, showing significantly poorer outcome for the latter of these hormonally driven subtypes (log rank p<0.05). Since the poorer outcome of luminal-B breast cancers may be due to their harboring more TP53 gene mutations (~34%), we used a gene expression signature reported for TP53 mutated estrogen receptor-positive (ER+) breast cancers (Coutant et al., 2011), and confirmed that the TCGA luminal-B cases significantly overexpress this signature relative to luminal-A breast cases in which TP53 mutations are uncommon (6%). Curiously, within each luminal subtype, while this signature correlated with TP53 mutations it did not correlate with TP53 loss of heterozygosity (LOH), which was also higher in luminal-B (~30%) relative to luminal-A (~10%) cases. We used the network analysis tool PARADIGM (Vaske et al., Bioinformatics 26, i247-245) to integrate both DNA copy number and transcriptome data and infer pathway activity and interaction differences between luminal-A and luminal-B cases, and between the luminal and basaloid subtypes. This tool merges features derived from curated signal transduction, transcriptional and metabolic pathways into a Superimposed Pathway (SuperPathway) containing ~3.1K unique activities, 1820 of which revealed significant differences among the breast cancer subtypes (Kruskal-Wallis test, Benjamin Hochberg FDR-corrected p<0.05). From the significant pathway activities differentiating basaloid from luminal-A (1399) or luminal-B (1122) subtypes, assessed using two independent approaches (functional enrichment/EASE scores, subnetwork analysis and hub interconnectivity >10 edges), higher FOXA1 (ER signaling) and lower HIF1A/ARNT transcription factor hub activities emerged as shared luminal differences relative to basaloid breast cancers. Of the 433 significant activities differentiating...
also indicated that RB1 and PTEN, two genes known to be involved in basal-like breast tumor formation, showed a particularly high incidence of these microevents. These microevents may alter expression of the involved gene as well, as suggested by data from microarray and mRNA-seq studies.

Conclusion: Using a high probe density, gene-centric aCGH microarray, we present evidence of small-scale genomic aberrations that may contribute to gene inactivation, and thus, genomic instability and tumor formation through a mechanism not detected using conventional copy number analyses.

PD03-07
Breast Cancer Heterogeneity and Treatment Resistance: Clues from Metaplastic Tumors.
Fielding-Habermann B, O’Sullivan DM, Longier M, MacDermed D, Fernandez-Santidrian A, Steele JB, Telli ML, Jeffrey SS, Murray S, Torkamani A, Cunliffe H, Vaughn SV. The Scripps Research Institute, La Jolla, CA; Scripps Clinic, La Jolla, CA; Stanford University, Stanford, CA; Translational Genomics Research Institute, Phoenix, AZ

At late stage, nearly all breast cancers are heterogeneous and refractory to treatment, like metaplastic breast cancer is at an early stage. These rare carcinomas are highly aggressive and de-differentiated. They are enriched for mesenchymal and stem cell features and essentially fail current therapies. As metaplastic tumors provide a time-compressed picture of breast cancer progression early on, understanding these tumors will yield insight into mechanisms that drive breast cancer into advanced stages and treatment resistance.

To investigate a genetic basis for heterogeneity in metaplastic breast cancer, we established a progression model comprising three cell lines. The cell lines were derived from a primary tumor, a local recurrence and a pleural effusion of a 40-year-old patient. The primary tumor was a stage III invasive metaplastic, triple negative, inflammatory breast cancer, resected after neoadjuvant chemotherapy (capecitabine and taxotere, then adriamycin and one cycle of bevacizumab). The local recurrence, biopsied seven months post mastectomy, developed after the patient received adjuvant carboplatin and gemcitabine for 3 cycles and then radiation to the chest wall. At this time, the patient had lung metastases and was treated with taxol and bevacizumab yielding a mixed response. Local invasive growth continued and a malignant pleural effusion developed four months later. Analyzing the genetic and molecular characteristics of this progression model in vitro, its tumorigenicity and metastasis in vivo, and interrogating lead findings in a growing collection of metaplastic tumors helps us to dissect the genetic heterogeneity in breast cancer, and potentially to identify the cell types that drive disease progression and treatment resistance.

Our gene expression analyses and genomic evaluations identified epithelial to mesenchymal transition (EMT) as a key characteristic in the progression and treatment resistance of this cancer. Major changes in cytoskeletal genes, chemokines and their receptors, amplification of drug transporter proteins, metalloproteinases and matrix proteins seen with increasing motility and invasiveness along with recruitment of host inflammatory responses in the in vivo model, loss of chromosomal regions harboring known and putative tumor suppressors, and deletions of genes encoding proteins for metabolic inactivation of sex hormones in the breast tissue, along with specific loss of clusters of desmosomal genes are guiding our understanding of metaplastic breast cancer progression. The results provide insight into the development, the extremely invasive nature, and treatment resistance of these tumors. Our collaborative network of clinicians, pathologists, translational genomic researchers and bioinformatics specialists will enable us to identify and prioritize genetic events as
disease drivers, prognostic biomarkers of disease progression, and determinants of treatment resistance. Our goal is to identify molecular and functional targets for effective therapy and evaluate them in the clinic. Lessons learned from metaplastic breast cancer will improve our understanding of breast cancer progression in general, and could translate into effective treatments for advanced breast cancer where current standard of care is failing.

**PD03-08**

BRCA1-Like Triple Negative Tumors: Clinicopathological Variables and Chemosensitivity to Alkylating Agents.

Wesseling J, Lips EH, Oonk AMM, Smits RM, van Rijn CCM, Mulder L, Laddach N, Savola SS, Wessels LFA, Nederlof PM, Rodenhuis S, Imholz ALT. Netherlands Cancer Institute-Antoni van Leeuwenhoek Hospital, Amsterdam, Netherlands; Deventer Hospital, Deventer, Netherlands; MRC-Holland, Amsterdam, Netherlands

Background

Our group has previously employed array Comparative Genomic Hybridization (aCGH) to assess the genomic patterns of BRCA1-mutated breast cancers. It is reasonable to assume that this pattern indicates ‘BRCA-ness’ and thus serves as a marker for homologous recombination deficiency. This BRCA1-like aCGH profile is also present in about half of all triple negative sporadic breast cancers and has been shown to be predictive for benefit from intensive chemotherapy with DNA crosslinking agents. To study BRCA1-like tumors and conventional dose chemotherapy sensitivity in more detail, we compared clinical factors and survival rates in a uniform cohort of triple negative breast tumors treated with alkylating agents.

Patients and methods

103 patients with triple negative tumors received conventional dose adjuvant chemotherapy with doxorubicin/cyclophosphamide. DNA was extracted from tumor samples and BRCA1-like profiles were assessed. Tumors were classified as BRCA1-like or non-BRCA1-like.

Standard clinical and histopathological factors were determined and compared between both groups. Relapse free survival (RFS), disease specific survival (DSS) and overall survival (OS) after diagnosis were compared between BRCA1-like and non-BRCA1-like tumors.

Results

66 tumors (65%) had a BRCA1-like profile, while 35 tumors (35%) did not show such a profile. Patients with BRCA1-like tumors tended to be younger and had more often node-negative disease compared to the patients with non-BRCA1-like tumors (p=0.058 and p=0.034, respectively). There was no significant difference in survival between BRCA1-like and non BRCA1-like patients after treatment with alkylating agents: the median RFS was 121 vs. 109 months, median DSS was 129 vs. 114 months and OS was 127 vs. 110 months, for BRCA1-like versus non-BRCA1-like tumors. T-stage was the only variable significantly associated with survival.

Conclusion

BRCA1-like tumors occurred in younger patients and were more often node negative, which are features shared with tumors in BRCA1-mutation carriers. We did not observe a difference in survival between BRCA1-like and non-BRCA1-like triple negative breast cancers after treatment with conventional dose chemotherapy with alkylating agents. These results confirm our previous findings that BRCA1-like tumors have similar sensitivity to anthracycline-based adjuvant chemotherapy as other triple-negative tumors. It will be important to establish whether BRCA1-like tumors also share the exquisite sensitivity of BRCA-mutated tumors to PARP-inhibitors.

**PD03-09**


Background: RNA biomarkers discovered by RT-PCR-based gene expression profiling of archival formalin-fixed paraffin-embedded (FFPE) tissue are the basis for very precise and sensitive clinical diagnostic tests, such as the 21 gene Oncotype DX® breast cancer assay. Both inherent limits of technical scalability and the small amounts of patient FFPE RNA available place practical constraints on the number of transcripts that can be interrogated by RT-PCR. We developed new methods for RNA profiling through massively parallel “next generation” sequencing (RNA-Seq) of archival FFPE specimens. We report here the technical performance of this methodology and compare the results to RT-PCR results obtained in one of the studies that were carried out to develop the 21 gene assay.

Methods: RNA was extracted in 2002 from 136 invasive breast tumors that were formalin-fixed and paraffin-embedded between 1990 and 1997. RNA-Seq was carried out using minor modifications to methods we have reported previously (Sinicropi et al., Advances in Genome Biology and Technology Conference, p. 170, 2010 and p. 198, 2011). Briefly, 0.1 mg of total RNA was selectively depleted of ribosomal RNA and sequencing libraries were prepared using a modification of the ScriptSeq™ kit from Epicentre. The libraries were sequenced on an Illumina HiSeq 2000 instrument with multiplexing of two libraries per lane for 50 cycles in one direction. The resulting FASTQ sequences were mapped to version hg19 of the human genome using the Illumina CASAVA pipeline. The total number of sequences (reads) that uniquely mapped to all exons of each RefSeq entry was used for quantification of expression levels.

Results: On average, there were 43 million reads per sample (range 31 – 58 million; SD=4.6 million) of which 69% uniquely mapped to the human genome. Ribosomal RNA was effectively removed and accounted for <0.3% of total counts. Significant coverage of a high proportion of the human genome was obtained, with 40% of RefSeq transcripts represented by a median of more than 100 reads. Using Cox proportional hazards analysis to evaluate the association of quantitative gene expression with breast cancer recurrence, the standardized hazard ratios and p-values for the 21 Oncotype DX genes determined by RNA-Seq were comparable to those originally obtained using RT-PCR. Moreover, whole transcriptome RNA-Seq identified more than 1800 new coding, intronic, and intergenic transcripts that strongly associated with breast cancer recurrence risk (at a false discovery rate <10%) and revealed heretofore unappreciated co-expressed gene networks. Summary: New methodology has been developed for application of next generation sequencing-based whole transcriptome profiling to small amounts of archival FFPE tissue. This technology has sensitivity and selectivity comparable to RT-PCR, can provide a vast increase in the number of interrogated transcripts, can reveal new biological relationships, and has excellent performance suitable for the discovery of RNA biomarkers.
PD03-10

Background: We and others have shown that high expression of proliferation and immune related genes are associated with pathological complete response (pCR) after neoadjuvant chemotherapy in breast cancer (BC). Here, we performed a meta-analysis to validate these findings and to interrogate the association between pCR and several other gene expression modules beyond standard clinico-pathological characteristics in BC subtypes.

Methods: We searched for publicly available gene expression studies evaluating anthracycline + taxane-based neoadjuvant chemotherapy. We identified 7 studies with complete genomic and clinico-pathological data including pCR totaling 788 patients. Relapse-free survival (RFS) data were available for 427 patients. We used gene expression data generated from pretreatment biopsies and computed 17 gene modules corresponding to proliferation-driven signatures, immune response, stroma activation, phosphatase and tensin homolog (PTEN) loss, chromosomal instability, and several other oncogenic pathways. We calculated odds ratios (OR) for pCR for one-unit increases in scaled modules, all adjusting for pretreatment clinico-pathological characteristics. Moreover, we tested for interactions between gene modules and ER or HER2 status for their association with pCR after adjusting for clinico-pathological characteristics. We used the false discovery rate (FDR) to adjust for multiple testing.

Results: We observed pCR in 178(22.6%) of 788 patients: 112(30.1%) of 372 with ER-/HER2- BC, 41(36%) of 113 with HER2+ BC, and 25(8.2%) of 303 with ER+/HER2- BC. High values of the proliferation-driven modules were associated with increased pCR rates in ER-/HER2- (e.g. Gene70 OR=2.34, 95%CI 1.22-4.59, p=0.01, FDR=0.10) and ER+/HER2- (e.g. Gene70 OR=3.26, CI 1.13-9.60, p=0.03, FDR=0.16), but not in the HER2+ subtype (e.g. Gene70 OR=0.82, CI 0.23-2.67, p=0.74, FDR=0.86). There was a trend for interaction between proliferation-driven modules (e.g. Gene70) and HER2 status (p=0.08, FDR=0.23). Interestingly, we demonstrated a strong association between high values of immune module and increased pCR rates in the HER2+ (OR=6.58, CI 2.20-23.40, p<0.01, FDR=0.03) and ER-/HER2- (OR=1.75, CI 1.12-2.76, p=0.02, FDR=0.10) but not in the ER+/HER2- subtype (OR=1.50, CI 0.63-3.42, p=0.35, FDR=0.49). Moreover, high values of AKT activation module were associated with decreased pCR rates in HER2+ (OR=0.38, CI 0.14-0.98, p=0.05, FDR=0.29) but not in the HER-/HER2- (OR=0.87, CI 0.56-1.37, p=0.56, FDR=0.68) or ER+/HER2- (OR=1.81, CI 0.65-5.30, p=0.27, FDR=0.47) subtype. Interactions between immune module and HER2 status and between AKT module and HER2 status were nominally significant (p=0.04 and p=0.05, respectively), but came with an FDR of 0.23. Of interest after a median follow-up of 3.47 years, (95% CI 3.18-3.70 years) patients with pCR had significantly longer RFS irrespective of BC subtypes (HR=0.20, 95% CI 0.08 to 0.50, p=0.001).

Conclusion: Different biological processes namely proliferation, immune response and AKT activation are associated with pCR in different BC subtypes. Our results suggest that new drugs that modulate immune responses in ER-/HER2- and HER2+ BC and target AKT activation in HER2+ BC might be evaluated in these subtypes.

PD04-01
Predictors of Recovery of Ovarian Function during Aromatase Inhibitor (AI) Therapy.
Henry NL, Banerjee M, Hayden J, Yakim E, Schott AF, Stearns V, Partridge AH, Hayes DF. University of Michigan Medical School, Ann Arbor; MI; Johns Hopkins School of Medicine; Dana Farber Cancer Institute.

Background: AIs may cause a paradoxical rise in estrogen levels due to re-activation of ovarian function in women with chemotherapy-induced ovarian failure (CIOF). Therefore, identification of residual ovarian estradiol production is critical if such women are treated with adjuvant AI therapy rather than tamoxifen. We performed a prospective registry trial to identify predictors of recovery of ovarian function during AI therapy.

Methods: Women with hormone receptor (HR) positive breast cancer who were pre- or peri-menopausal at diagnosis and who remained amenorrheic for ≥ 28 weeks after cyclophosphamide-containing adjuvant chemotherapy were enrolled in a multi-institutional, open-label clinical trial of anastrozole (1 mg/day). Following confirmation that serum estradiol (E2) levels were <20 pg/ml using an ultrasensitive E2 assay (Quest Diagnostics), subjects initiated anastrozole. Serum E2 was assessed biweekly for 12 weeks, then less frequently, for 72 weeks. Multivariable logistic regression was used to evaluate clinical predictors (age at AI initiation OR chemotherapy, menopausal status at chemotherapy, body mass index (BMI), baseline E2) of recovery of ovarian function defined as elevated serum E2 levels or return of menses.

Results: Sixty-nine women were enrolled; current status is given in Table 1.

Median age at initiation of chemotherapy was 47.2 yrs (range 37-55), median time since chemotherapy was 0.8 yrs (range 0.3-6.4), and median age at enrollment was 49.8 yrs (range 40-58). Thirty-six had received tamoxifen. We observed elevated E2 concentrations or return of menses during AI therapy in 21 subjects after a median 2.0 mo (range 0.6-17); for that cohort, median age at chemotherapy was 43.8 yrs (range 37-51) and median age at AI initiation was 45.8 yrs (range 40-56). In contrast, for the 15 subjects who had postmenopausal E2 levels for at least 48 wks, median age at chemotherapy was 49.2 yrs (range 44-52) and median age at AI initiation was 50.7 yrs (range 44-55). Age at chemotherapy (p=0.0006) and age at AI initiation (p=0.001) were statistically significant different between the 2 cohorts. On multivariable analysis, age at chemotherapy and age at AI initiation remained significant when each was adjusted for menopausal status, BMI, and baseline E2 (odds ratio (OR) 1.64, p=0.0102 and OR 1.47, p=0.015, respectively).

Conclusions: A significant proportion of women who develop CIOF recover ovarian function during AI therapy. Although recovery is usually rapid, it can occur at least one year following initiation of AI therapy. Younger age was the strongest predictor of recovery of ovarian function, although 2 of 21 women who developed elevated E2 levels or return of menses were older than 50 yrs at the time of chemotherapy. Tamoxifen remains the standard of care for women with CIOF; if use of an AI is necessary, patients should be monitored frequently with high-quality E2 assays for recurrent ovarian function.
**PD04-02**

Recovery of Ovarian Function in Breast Cancer Patients with Chemotherapy-Induced Amenorrhea Receiving Anastrozole in the Dutch DATA Study.

Tjjan-Heijnen VC, Smorenburg CH, de Graaf H, Erdkamp F, Honkoop A, Wals J, van Gastel S, van der Sangen M, Seynaeve C, Nortier JW, Borm G. Maastricht University Medical Centre, Netherlands; Medical Centre Alkmaar, Netherlands; Medical Centre Leeuwarden, Netherlands; Orbis Medical Centre, Netherlands; Isala Clinics, Netherlands; Atrium Medical Centre, Netherlands; Comprehensive Cancer Centre Netherlands Nijmegen, Netherlands; Catharina-Hospital, Netherlands; Erasmus University Medical Centre, Netherlands; Leiden University Medical Centre, Netherlands; Radboud University Nijmegen Medical Centre, Netherlands

**Background:** In early stage hormone receptor positive breast cancer, aromatase inhibitors (AIs) are established as adjuvant therapy for postmenopausal women. In daily practice AIs are also offered to patients with chemotherapy-induced amenorrhea (CIA). The impact of AIs on estrogen (E2) levels in these patients has not extensively been studied, although this could be very relevant for the efficacy and safety of the adjuvant hormonal treatment. The Dutch phase III DATA study is assessing the impact on disease-free survival of 3 vs. 6 years of anastrozole after 2-3 years of tamoxifen (N=1900 patients in total), and has included both postmenopausal patients and patients with CIA. The current analysis reports on the hormonal data in the CIA group.

**Patients and methods:** We identified patients from the DATA study < 55 years of age at randomization who had received adjuvant chemotherapy and developed CIA, and excluded patients with ovariectomy or use of LHRH agonist. Patients were considered as having CIA if they were in amenorrhea since 3 months before start of chemotherapy up to 6 months after start of chemotherapy, and did not resume menses during tamoxifen therapy. Patients were eligible if postmenopausal E2 levels were confirmed within the last three months before randomization. Plasma FSH and E2 levels were serially determined at 6-month intervals.

**Results:** A total of 285 patients with CIA were identified in the DATA study. Median age was 50.8 years (range 35.9 - 54.9). Results on E2 and FSH levels are presented in the Table. During treatment with anastrozole, FSH levels tended to increase over time and E2 levels didn’t decline. Of note, FSH increased in nearly all patients with significantly elevated (premenopausal) E2 levels, in contrast to the pattern seen in spontaneous recovery of ovarian function. During follow-up, 4 patients had vaginal bleeding, 2 of them having postmenopausal E2 levels. In 8 (2.8%) patients E2 levels became > 200 pmol/l (considered premenopausal) after 12-30 months use of AI. Using a more strict cutoff value of E2 (≥ 100 pmol/l), 62 (21.8%) patients had elevated levels of E2 during AI treatment. With 70 pmol/l as cutoff value, 117 (41.0%) patients had at some point during treatment an increased E2 level. Updated and detailed analyses will be presented at the meeting.

<table>
<thead>
<tr>
<th>No. of patients</th>
<th>E2 median (pmol/l)</th>
<th>E2 range (pmol/l)</th>
<th>No. pts with E2 &gt; 200 pmol/l</th>
<th>No. pts with E2 &gt; 100 pmol/l</th>
<th>FSH median (U/l)</th>
<th>FSH range (U/l)</th>
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<tbody>
<tr>
<td>Baseline</td>
<td>285</td>
<td>8-192</td>
<td>1</td>
<td>30</td>
<td>1-78</td>
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<tr>
<td>T=6 months</td>
<td>111</td>
<td>8-190</td>
<td>1</td>
<td>26</td>
<td>1-75</td>
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<tr>
<td>T=12 months</td>
<td>121</td>
<td>8-700</td>
<td>3</td>
<td>14</td>
<td>81</td>
<td></td>
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<td>112</td>
<td>11-463</td>
<td>2</td>
<td>12</td>
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</tr>
<tr>
<td>T=24 months</td>
<td>81</td>
<td>18-390</td>
<td>2</td>
<td>7</td>
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<td>62</td>
<td>12-219</td>
<td>1</td>
<td>9</td>
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</tbody>
</table>

**Conclusion:** In this first series of a large number of CIA patients with available data on E2 and FSH levels during anastrozole therapy, we observed high E2 levels in a substantial number of patients. The combination of increased E2 and FSH levels may indicate continuous stimulation of remaining ovarian follicles. The efficacy of AIs in women with CIA without strict E2 monitoring and adequate treatment modification in the presence of increasing E2 can be questioned. Further data hereon are warranted.

**Supported by:** AstraZeneca NL and the Dutch Breast Cancer Trials’ Group (BOOG).

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**PD04-03**


**Background:** Sexual dysfunction is reported after chemotherapy and endocrine therapies. However, the prevalence and severity of sexual dysfunction in premenopausal women undergoing therapy for both local and metastatic disease is not well defined. This study was performed in order to understand the impact of contemporary breast cancer treatment on the prevalence and severity of sexual health in premenopausal women.

**Methods:** We developed a survey that includes a previously validated questionnaire, the Female Sexual Function Index (FSFI), as well as an established measure of health-related quality of life (the EuroQol EQ-5D), and disease-specific items to characterize sexual dysfunction and its causes based on literature review and expert consultations. Anonymous administration of the surveys was conducted in outpatient clinic waiting areas of the Breast Cancer Center at Memorial Sloan-Kettering Cancer Center (MSKCC), under an IRB waiver of consent.

**Results:** 372 consecutively approached premenopausal women with breast cancer of any stage, undergoing treatment were each queried once. The mean age was 47. 87% reported current or past hormonal treatment, and 86% reported current or past chemotherapy (76% adjuvant; 24% for metastatic disease). Sexual dysfunction attributed to breast cancer or its treatment, defined as an FSFI score <26, was reported by 75% of respondents with a mean score of 16.3. Among these women, 79% of patients considered their sexual symptoms to be bothersome, with 51% noting moderate or severe levels of bother (score >=5/10). In a multivariate analysis, metastatic disease, development of amenorrhea from cancer treatment, antidepressant use and poorer overall health were each significantly associated with worse FSFI scores. Lower FSFI scores were also significantly associated with worse health-related quality of life.

**Conclusion:** Sexual dysfunction is prevalent in premenopausal women treated for breast cancer and should be discussed with patients as a potential adverse effect of therapy. Assessment of sexual symptoms throughout treatment and beyond may facilitate the use of potential interventions such as lubricants, dilators, treatment modification and counseling.

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**PD04-04**

Sexual Dysfunction in Women with Early Stage Breast Cancer on Endocrine Therapy: Encouraging Results from a Prospective Study.

Frechette D, Paquet L, Verma S, Clemons M, Wheatley-Price P, Gertler SZ, Song X, Graham N, Dent S. The Ottawa Hospital Cancer Centre, Ottawa, ON, Canada; Carleton University, Ottawa, ON, Canada

**Background:** While the side effects of endocrine therapy (ET) for early stage breast cancer (EBC) have been extensively studied, the link between ET and sexual dysfunction (SD) remains a contentious issue. Most studies have focused on documenting only the presence of problems in specific domains of endocrine symptoms (ES) (e.g.
hot flushes, vaginal dryness) and sexual functioning (SF) (interest, satisfaction, arousal, lubrication) without also taking sexual distress into account. To our knowledge, there have been no prospective longitudinal studies evaluating SF and SD before the onset of ET and after treatment initiation. We report the initial 6 month results of this study of SD in women initiating ET for EBC.

Methods: Hormone receptor positive EBC post-menopausal women were approached for a larger study of SF aimed at comparing the prevalence of SD across endocrine agents (tamoxifen vs aromatase inhibitor) and at evaluating the impact of anxious predisposition and ES on SD. Here we report on changes in ES, SF and SD after 6 months of ET. SF was evaluated with the Female Sexual Function Index (FSFI) while sexual distress was assessed with the Female Sexual Distress Scale. ES were measured with FACT-B ES subscale. Participants completed questionnaires prior to initiation (T0) of ET and at 6 months (T1). SD was assessed using the APA classification.

Results: Between January 2009 and May 2011, 118 EBC patients entered the study and 83 have completed both assessments (mean age 62: 30% received chemotherapy). Over time, the levels of ES increased (p < 0.001). Despite the worsening of ES at T1, no decline in SF was observed, this for each FSFI domain (desire, arousal, lubrication, discomfort during intercourse and satisfaction). There was no change in the percentage of women reporting 1 or more sexual problems over time (85% vs 87%, ns) nor in the percentage who were sexually distressed (32% vs 34%, ns). The prevalence of SD did not increase after 6 months of ET (T0=28% vs T1=33%, ns). There were no differences in the percentage of women who worsened (i.e., no SD at T0 but SD at T1, 12%) and those who improved (SD at T0 but no SD at T1, 7%) over time (McNemar X², p >.5) Importantly, women classified as experiencing SD at T0 were more likely to also experience SD at T1 (OR=4.5, 95% CI=2.162 to 9.366) than women who had no SD at T0.

Discussion: This is the first prospective case cohort study evaluating ES, SF and SD in women with EBC on ET. The good news for women is that although ES increased during ET (p < 0.001), this did not have a negative impact on sexual problems (85% vs 87%, ns) or SD (32% vs 34%, ns). This is encouraging news but longer follow-up of these women will provide further insight into the impact of ET on ES and SD over time (>6 months). The impact of specific types of ET on ES, and SD will also be evaluated. Of interest, the high uptake and high completion rate (>80%) of questionnaires, indicate this is a matter of relevance and importance to women taking adjuvant ET and merits acknowledgement and sensitive discussion.

PD04-06


Rosenberg SM, Tamimi RM, Gelber S, Kereakoglow S, Borges V, Come S, Schapira L, Winer E, Partridge A. Harvard School of Public Health, Boston, MA; Dana-Farber Cancer Institute, Boston, MA; University of Colorado Cancer Center, Denver, CO; Beth Israel Deaconess Medical Center, Boston, MA; Massachusetts General Hospital, Boston, MA

Background: While there is evidence that younger women with breast cancer are more likely to experience compromised quality of life compared to older women, few studies have prospectively explored the impact of treatment, including surgery, chemotherapy, and hormone therapy, on body image, in particular, in very young women (≤40 years old). This analysis examined treatment-associated differences in self-reported body image among a large cohort of young women diagnosed with breast cancer. Methods: 431 women enrolled in an ongoing multi-center prospective cohort study with Stage 0-Stage III breast cancer were included in this analysis. Body image was measured at baseline (1-12 months following diagnosis) using three items from the Cancer Rehabilitation Evaluation System (CARES) survey. CARES scores range from 0-4, with higher scores indicative of greater image concerns. Mean differences in CARES scores between treatment groups (chemotherapy within the last month vs. none; hormone therapy vs. none; lumpectomy vs. mastectomy alone vs. mastectomy + reconstruction) were estimated using T-tests and one-way ANOVA. To control for concurrent treatment, stage, and time since diagnosis, multiple linear regression models were fit and least squares means estimated and compared between treatment groups. Multiple comparisons were adjusted for using the Bonferroni correction. Results: Median age at diagnosis was 37 (range: 17-40) and median time from diagnosis to study enrollment was 5 months (range: 1-12 months). In the unadjusted analysis, there were no significant differences in scores between women who had received chemotherapy within the last month and those who did not (p=0.80), while women who reported hormone treatment had higher mean CARES scores compared to women who did not (p=0.04). Among women who had undergone surgery (n=370), women who had lumpectomies had a mean CARES score of 0.95, which was significantly lower (p<0.0001) compared to both women who had undergone mastectomy alone (CARES: 1.89) and women who reported mastectomy + reconstruction (CARES: 1.53). After adjusting for concurrent treatment (including radiation), time since diagnosis, and stage of disease, only differences between surgical groups remained significant (p<0.0001), with mean scores among women who had either undergone mastectomy alone (CARES: 2.02) or together with reconstruction (CARES: 1.58) higher compared to those who had a breast conserving procedure (CARES: 0.92)

Conclusion: To the best of our knowledge, this is the largest analysis of treatment-related body image issues in young women with breast cancer. Treatment with chemotherapy and hormonal therapy did not appear to affect short-term body image. However, women who had a breast conserving procedure had the fewest body image concerns as measured by the CARES, while women undergoing more radical surgery appear to be at increased risk for low perceived body image though this may be mitigated to a degree by reconstruction. Further analyses will explore whether differences between surgical groups persist over time as well as examine the trajectory of change over the course of follow-up.

PD04-06

A Randomised Controlled Trial of Quality of Life and Fatigue after Support Group Intervention in Primary Breast Cancer Patients.

Granstam Björneklett H, Lindemalm C, Ojutkangas M-L, Berglund A, Letocha H, Strang P, Bergkvist L. Centre for Clinical Research, Västerås, Sweden; Cancer Centre Karolinska, Solna, Sweden; Centre for Clinical Research, Central Hospital, Västerås, Sweden; Karolinska Institute, SSH, Stockholm, Sweden; Karolinska Institute, Stockholm, Sweden

Background: When diagnosed with breast cancer, most women’s lives change as well as their perspectives on and appreciation of life. The aim of the present study was to evaluate whether psychosocial support intervention could influence health-related quality of life and fatigue during the first year after diagnosis.

Material and Methods: Of 1382 patients with newly diagnosed breast cancer, 191 + 191 patients were randomized to an intervention group or to a routine control group respectively.
The intervention group received support intervention that lasted one week on a residential basis, followed by four days of follow-up two months later. The support intervention included informative-educational parts, relaxation training, mental visualisation and non-verbal communication. Health-related quality of life was measured using the EORTC-QLQ 30 and QLQ-BR23 questionnaires and fatigue with the Norwegian version of the Fatigue scale at baseline, 2, 6 and 12 months after intervention.

Result: There were a time-dependent improvement in both functional and symptom scales between baseline and 12 months as measured by the EORTC QLQ 30 and BR 23 questionnaires and there was a decrease in fatigue between baseline and after 2 months with further improvement up to 12 months in both groups but there were no differences between the intervention and control groups at any point in time.

Conclusion: Health related Quality of Life improves and symptoms of fatigue decreases over time, but we could not see any additional effect from the rehabilitation program in this setting.

Key words: Support intervention, breast cancer, health-related quality of life, fatigue, EORTC-QLQ 30 and BR 23, the Norwegian version of the fatigue scale.

Cognitive Function and Reproductive Hormones in Women Receiving Anastrozole.

Bender CM, Sereika SM, Ryan CM, Berga SL. University of Pittsburgh, Pittsburgh, PA; Emory University, Atlanta, GA

Background: The effects of adjuvant hormonal therapy on hormone levels may contribute to deterioration in cognitive function experienced by women with breast cancer. Estrogen receptors are present throughout the central nervous system. Estrogen binding increases ChAT, synaptogenesis and dendritic spine density in the hippocampus and hypothalamus and decreases monoamine oxidase activity. Aromatase inhibitors, such as anastrozole, interrupt estrogen biosynthesis resulting in profound estrogen reductions. We studied whether changes in reproductive hormone levels mediate changes in cognitive function in 3 cohorts of postmenopausal women; women with breast cancer who receive chemotherapy+anastrozole (CA; n=41) or anastrozole only (AO; n=50) or healthy women (n=44).

Methods: We assessed cognitive function and reproductive hormones (E2, LH, FSH) before therapy and at 6, 12 and 18 months post-therapy initiation. A battery of neuropsychological measures was used to assess multiple cognitive domains. Using mixed effects modeling, we analyzed changes in hormone levels from pretreatment to 6, 12 and 18 months post-therapy initiation and then explored intercorrelations between changes in hormone levels and cognitive function at all timepoints.

Results: Women were an average 59.3 years of age with an average 14.9 years of education. No significant group-by-time effects were found for LH. However, we found significant group by time effects for E2 when comparing CA with controls (p=.0002) and AO with controls (p=.0001) and for FSH when comparing AO with controls (p=.03). We found that E2 declined from pretreatment in the breast cancer groups as follows; CA [E2 declined from pretreatment to immediately post-chemotherapy (p = .09), and at 6 (p = .0002) and 12 (p = .014) months post-anastrozole initiation, and AO [E2 declined from pretreatment to 6 (p < .0001), 12 (p = .004) and 18 (p = .0001) months post-anastrozole initiation]. We also found increases in FSH in the AO group from pretreatment to 6 (p = .002) and 12 (p = .05) months. No significant within-group changes for E2, FSH, or LH were observed for controls. For the full sample, the intercorrelations revealed that reductions in E2 were related to poorer psychomotor efficiency from baseline to 18 months post-baseline (r = .358, p = .02). For the AO group, reductions in E2 were related to poorer executive function (r = .600, p = .002) from 6 to 12 months post-anastrozole initiation and poorer psychomotor efficiency (r = .453, p = .07) from pretreatment to 18 months post-anastrozole initiation. For the CA group, reductions in E2 were marginally significantly related to poorer attention (r = .307, p = .08) from pretreatment to post-chemotherapy; and to poorer executive function from pretreatment to 6 months (r = .446, p = .06) and 12 months (r = .651, p = .03) post-anastrozole initiation. No significant relationships between changes in E2 levels and cognitive function were found in the controls.

Conclusions: Reductions in E2 may be related to cognitive deterioration in women with breast cancer. Further examination of these relationships is needed to confirm the results and determine whether these relationships persist through the remainder and after the conclusion of therapy.


Cimprich B, Hayes DF, Asken MK, Jung MS, Berman MG, Therrien B, Reuter-Lorenz PA, Zhang M, Pelletier S, Noll DC. University of Michigan, Ann Arbor, MI

Background: The underlying brain mechanisms of altered cognitive function associated with adjuvant chemotherapy for breast cancer, commonly designated “chemobrain”, have yet to be determined. Recent research indicates that compromised cognitive function may already exist prior to treatment, adding to the complexity of determining the true impact of adjuvant chemotherapy on brain function. We examined whether two common symptoms associated with breast cancer diagnosis, worry and fatigue, might influence neurocognitive responses during functional magnetic resonance imaging (fMRI) before adjuvant therapy.

Methods: Seventy-five women (29 -75 years) awaiting either adjuvant chemotherapy (n=25) or radiation therapy (n=25) for localized breast cancer and age-matched controls without breast cancer (n=25) were enrolled. Participants performed a verbal working memory task (VWMT) with varying levels of demand for cognitive control during fMRI scanning and provided self-reports of worry and fatigue after scanning. Imaging data were analyzed using the general linear model implemented in SPM5; comparative statistics were used to determine group differences in self-report and behavioral measures.

Results: In general, the pre-chemotherapy group showed compromised cognitive functioning relative to the other groups. Specifically, they reported higher worry than the pre-radiation therapy (p = .036) and control (p = .047) groups. The pre-chemotherapy group also identified greater fatigue than controls (p = .04); the pre-radiation group reported a fatigue level between the other two groups. In the VWMT, the pre-chemotherapy group was less accurate than controls (p = .05), while the mean accuracy for the pre-radiation group again fell between these two groups. fMRI results showed that the control group selectivity increased recruitment of executive control brain regions including the left inferior frontal gyrus (LIFG) and the anterior cingulate cortex (ACC) in response to high vs. low demand conditions. However, recruitment of LIFG and ACC by the control
group was greater than that by both the pre-chemotherapy (p = 0.01 and p = 0.03 respectively) and the pre-radiation groups (p = 0.07 and p = 0.02 respectively). Overall, higher fatigue was correlated with failure to increase recruitment of the LIFG and ACC in high vs. low demand conditions (p = 0.002 and p = 0.078 respectively). Greater worry was correlated with failure to suppress task-relevant activation of default network regions, particularly the posterior cingulate cortex, in high vs. low demand conditions, and with lower accuracy in the VWM task (p = 0.01). Thus, fatigue and worry made independent contributions to altered neurocognitive responses prior to any adjuvant treatment.

Discussion: These findings suggest that women awaiting adjuvant chemotherapy are vulnerable to compromised cognitive functioning, specifically in the domains of attention and working memory, related to higher worry and fatigue. Worry and fatigue may contribute to the cognitive impact often attributed to adjuvant chemotherapy. Therapeutic interventions to counteract worry and fatigue hold potential for optimizing cognitive function prior to adjuvant treatment for breast cancer.

PD04-09
Self-Reported Cognitive Attributes and Fatigue Improve over Long-Term Follow-Up in Breast Cancer Survivors; Some Cognitive Attributes Are Worse in Breast Cancer Patients Than in Non-Cancer Controls.
Hsu T, Ennis M, Hood N, Goodwin PJ. University of Toronto, Toronto, ON, Canada; Applied Statistician, Markham, ON, Canada; Mount Sinai Hospital, Toronto, ON, Canada; Mount Sinai and Princess Margaret Hospitals, Samuel Lunenfeld Research Institute, University of Toronto, Toronto, ON, Canada

Background: Cognitive deficits and fatigue have been reported in breast cancer (BC) patients undergoing a variety of treatments, including chemotherapy; in some studies these concerns persist after completion of treatment. We examined these factors over time (median 11 years) in a longitudinal study of BC patients and compared the status of long-term BC survivors to non-BC controls.

Methods: 535 T1-3, N0-1, M0 BC patients were enrolled 1989-96; 260 women survived without metastases to participate in long-term follow-up (LTFU) measurements in 2006-8. 161 controls without BC were enrolled 2007-8. Questionnaires examined a range of QOL attributes; here we focus on items related to cognition and fatigue, including: EORTC QLQ C30 (cognitive functioning, fatigue), Profile of Mood States (POMS - confusion/bewilderment, fatigue/inertia, vigor/activity), Fatigue Symptom Inventory (FSI - average fatigue past week, total disruption index), Everyday Problems (EDP - forgetfulness, difficulty concentrating, easily distracted). 166 of the BC patients had also completed EORTC and POMS at diagnosis and 1 year, and change in items on these questionnaires was calculated. Age and income adjusted differences between BC (LTFU) and controls were analyzed. Clinical significance was defined as ≥5% of the scale range or an effect size of ≥0.2 and statistical significance as p < 0.05.

Results: BC cases were older than controls (62.3 vs 59.1 years), had lower education (24.6 vs 10.2% high school only) and family income (38.2 vs 19.8% < $60,000 annually) (all p < 0.01). Cognitive attributes: BC patients showed clinically and statistically significant improvements between diagnosis and LTFU on POMS confusion/bewilderment but not EORTC cognitive functioning. Comparing BC (LTFU) to controls, in multivariate analyses adjusted for age and income, EORTC cognitive functioning (81.5 vs 87.4) and EDP forgetfulness (0.9 vs 0.6) were clinically and statistically significantly worse in BC patients than controls. In contrast, POMS confusion/bewilderment, EDP difficulty concentrating and EDP easily distracted scores were similar in BC patients at LTFU and controls. Fatigue: BC patients showed clinically and statistically significant improvements between diagnosis and LTFU on EORTC fatigue, POMS fatigue/inertia and POMS vigor/activity. After adjustment for age and income there were no clinically and statistically significant differences between BC (LTFU) and controls on any fatigue items.

Conclusions: Fatigue items, as well as some cognitive items, improved over time in BC patients and scores were comparable to scores in non-BC controls at LTFU. However, scores on some cognitive items were worse in LTFU BC patients than in non-BC controls raising concerns that BC diagnosis and treatment may be associated with long-term adverse effects on some aspects of self-reported cognitive attributes.

(Funded by The Breast Cancer Research Foundation)

PD05-01
Guarneri V, Frassoldati A, Ficarra G, Maiorana A, Bettelli S, Bottini A, Cagossi K, Bisagni G, Ravaoli A, Amadori M, Musolino A, Cavanna L, Orlando L, Giardina G, Piacentini F, Bagnalasta M, Conte P. Modena University Hospital, Modena, Italy; Medical Oncology, Ferrara University Hospital; Modena University Hospital; Istituti Ospitalieri, Cremona; Ravazzini Hospital, Carpi; Arcispedale Santa Maria Nuova, Reggio Emilia; Ospedale Infermi, Rimini; Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori, Meldola (FC); Parma University Hospital; Hospital of Piacenza; Ospedale di Circolo e Fondazione Macchi, Varese; Perrino Hospital, Brindisi; GlaxoSmithKline, Verona

Introduction: The CHER-Lob study is a randomized phase II trial of preoperative sequential taxanes-anthraclycines in combination with trastuzumab, lapatinib, or combined trastuzumab and lapatinib in HER2 positive, stage II-III breast cancer patients. A translational program to evaluate predictors of response as well as treatment effects on tissue biomarkers was pre-planned.

Methods: The CHER-Lob translational program includes the central evaluation of HER2, p95HER2, PTEN, pAKT, Ki67 on pre and post-therapy samples. All these biomarkers have been evaluated by immunohistochemistry. FISH analysis was performed in case of HER2 IHC 2+, and in all the discordant cases between central and local laboratories. Biomarkers change from baseline to surgery has been evaluated with the Wilcoxon signed-ranks matched-pair test.

Results: 121 patients have been randomized. The pathologic complete response rate (breast and axillary lymphnodes) was 26% in Arm A (chemotherapy + trastuzumab), 28% in arm B (chemotherapy + lapatinib) and 44% in arm C (chemotherapy + trastuzumab and lapatinib). The concordance between central and local HER2 assessment on pre-treatment biopsy was 97%. The mean (min;max) PTEN expression pre- and post-therapy were 66% (0;100) and 68.4% (0;100) respectively. The mean (min;max) pAKT expression pre- and post-therapy were 23.3% (0;100) and 8.8% (0;90) respectively. A significant decrease was observed in the overall samples (p=0.01). When analyzing the lapatinib alone arm, the difference was no longer significant (p=0.06), while it maintained significance when evaluating the two trastuzumab containing arms (p=0.0013). The mean (min;max) ki67 expression pre- and post-therapy were 29.5% (4;90) and 16.6% (1;50) respectively. A significant decrease was observed when looking at the...
whole population ($p<0.0001$). A significantly higher ki67 inhibition was observed in the dual vs single anti-Her2 therapy ($p=0.003$). **Conclusions:** The central HER2 retesting showed a high concordance with local laboratories. Treatment induced a suppression in pAKT expression, that was higher in patients receiving trastuzumab. The dual anti-Her2 blockade induced a higher Ki67 inhibition as compared to single anti-Her2 blockade. The evaluation of the predictive and prognostic role of these biomarkers is ongoing. Supported by GlaxoSmithKline

**PD05-02**

Effect of HER2/Topoisomerase II alpha (TOP2A) Gene Status or Protein Expression and Chromosome 17 (CEP17) Polysomy on the Outcome of Breast Cancer Patients Treated with Anthracycline-Containing Dose-Dense Sequential Adjuvant Chemotherapy with or without Paclitaxel – A Pooled Analysis of Two Hellenic Cooperative Oncology Group (HeCOG) Phase III Trials. Dafni U, Bobos M, Tsoiki E, Batistatou A, Kolesta F, Televantou D, Gogas H, Linardou H, Pectasides D, Kalogeras KT, Galani E, Koutras A, Papadimitriou CA, Fountzilas G. Hellenic Cooperative Oncology Group (HeCOG), Athens, Greece

**Background:** The HER2 gene has been established as a valid biomarker in the treatment of breast cancer patients with trastuzumab and probably with other agents, such as paclitaxel or anthracyclines. The TOP2A gene has been associated with response to anthracyclines. The relationship of HER2/TOP2A gene status in the presence of CEP17 polysomy with patients’ outcome following adjuvant treatment with anthracyclines with or without paclitaxel is not established.

**Patients and methods:** Formalin-fixed paraffin-embedded (FFPE) tumor tissue blocks from 1,033 patients (61.5% of 1,681 randomized patients) with high-risk operable breast cancer enrolled in two sequential phase III trials were assessed in a central laboratory for HER2/TOP2A gene amplification and CEP17 polysomy by fluorescence in situ hybridization (FISH) and tumors were categorized according to the 2007 American Society of Clinical Oncology/College of American Pathologists guidelines. HER2 and TOP2A amplification was defined as a gene/CEP17 ratio of $\geq$2.2 and $>=$2.0, respectively or a gene copy number of $>6$. Additionally, HER2, TOP2A, ER/PgR, Ki67, CK5 and EGFR protein expression were assessed by immunohistochemistry (IHC) and all patients were classified according to their IHC phenotype. Treatment consisted of epirubicin-based dose-dense sequential adjuvant chemotherapy followed by hormonal therapy and radiation, as indicated.

**Results:** Disease-free survival (DFS) and overall survival (OS) did not differ significantly between treatment groups. Median follow-up was 92 months, while 5-year DFS (OS) rates were 74% (88%), 69% (81%) and 75% (86%) for the E-T-CMF, E-CMF and ET-CMF groups, respectively. HER2 amplification was found in 24.1% of the patients and TOP2A amplification in 10.3%. In total, 46.7% of HER2 amplified tumors demonstrated TOP2A co-amplification. The median (range) of HER2, TOP2A and CEP17 copy numbers was 2.55 (0.70-45.15), 2.2 (0.50-26.15) and 2.05 (0.45-26.55), respectively. 21% of the tumors were considered to be polysomic (32.5% of those with HER2 amplification). Adjusting for treatment groups in the Cox model, TOP2A amplification, CEP17 polysomy and HER2/TOP2A co-amplification were not associated with either DFS or OS. Treatment with paclitaxel was associated with improved survival in the HER2-amplified subgroup (HR=0.493, interaction $p=0.036$; adjusting for clinicopathological prognostic factors: HR=0.553, interaction $p=0.054$), an association that was not apparent for DFS. Conclusions: HER2 amplification was predictive for OS benefit from adjuvant treatment with paclitaxel in patients treated with epirubicin-based dose-dense sequential adjuvant chemotherapy, but not for DFS. TOP2A amplification, CEP17 polysomy and HER2/TOP2A co-amplification were not associated with outcome.


**PD05-03**

Impact of Quantitative Measurement of HER2, HER3, HER4, EGFR, ER and PTEN Protein Expression on Benefit to Adjuvant Trastuzumab in Early-Stage HER2+ Breast Cancer Patients in NCCTG N9831. Perez EA, Ballman KV, Reinholz MM, Dueck AC, Cheng H, Jenkins RB, McCullough AE, Chen B, Davidson NE, Martino S, Kaufman PA, Kutheh LA, Sledge GW, Geiger KJ, Ingle JN, Tenner KS, Harris LN, Gradow JR, Rim DL, Mayo Clinic, Jacksonville, FL; Mayo Clinic, Rochester, MN; Mayo Clinic, Scottsdale, AZ; University of Pittsburgh Cancer Institute, Pittsburgh, PA; Angeles Clinic and Research Institute, Santa Monica, CA; Dartmouth Hitchcock Medical Center, Lebanon, NH; Oncology Associates of Cedar Rapids, Cedar Rapids, IA; Indiana University Medical Center Cancer Pavilion, Indianapolis, IN; Yale University, New Haven, CT; Seattle Cancer Care Alliance, Seattle, WA

**Background:** Prediction of benefit from trastuzumab in patients (pts) with HER2+ breast cancer remains an important goal. We sought to investigate the predictive value of quantitative measurement of HER2, HER3, HER4, EGFR, ER and PTEN protein expression on the benefit of trastuzumab in the phase III HER2+ adjuvant N9831 study for pts randomized to chemotherapy alone (Arm A) or chemotherapy with sequential (Arm B) or concurrent trastuzumab (Arm C).

**Methods:** For each marker, we evaluated quantitative expression, relationship with demographic data, and association with disease-free survival (DFS) of pts. Freshly cut tissue microarray slides with up to three-fold redundancy per specimen from the N9831 cohort were treated identically using the AQUA (Camp, et al; Nat Med 2002, JCO 2008) method of quantitative immunofluorescence for each marker. HER2 was tested with CBB11 (mouse monoclonal, Biocare, Inc.) and preliminary results were available for 698 of nearly 1400 pt specimens to be tested. The minimum value per pt was used in statistical analysis. Specimens were classified with high versus low expression based on a median value cutoff for each marker. Median follow-up was 7.0 yrs.

**Results:** Quantitative HER2 was compared with centrally performed HER2 testing by IHC and FISH. Median quantitative HER2 via AQUA was 10,017 units for the HER2 IHC 3+ group (n=607) versus 1054, 831, and 970 for the HER2 IHC 2+ (n=68), 1+ (n=11), and 0 (n=11) groups, respectively. The Spearman correlation between quantitative HER2 and FISH HER2/CEP17 ratio was 0.32 ($p<0.001$). High quantitative HER2 was associated with lower percentage of hormone receptor positivity (48% vs 59%, chi-sq $p=0.003$) but not associated with age, race, nodal positivity, tumor histology, grade, or size. High HER2 did not impact DFS in any arm of the study (See Table). Data for additional HER2 testing, HER3, HER4, EGFR, ER and PTEN are in process and will be ready by September, 2011.

**Conclusions:** Similar to results based on standard HER2 testing by IHC and FISH in N9831, quantitative HER2 did not impact benefit from adjuvant trastuzumab. Results for additional markers will be presented. Our complete quantitative results for a second epitope on HER2 and FISH HER2/CEP17 ratio in N9831 are presented. Our complete quantitative results for a second epitope on HER2 and FISH HER2/CEP17 ratio in N9831 are presented.
HER2, HER3, HER4, ER and EGFR will be the first report of these markers in a large patient cohort in the adjudant setting.

### PD05-04
**Quantitative Measurement of Antigen Degradation in NCCTG N9831 Tissue Microarrays.**
Cheng H, Rimm DL, Reinholz MM, Lingle WL, Ballman KV, Dueck AC, Chen B, McCullough AE, Jenkins RB, Perez EA. Yale University School of Medicine, New Haven, CT; Mayo Clinic, Rochester, MN; Mayo Clinic, Scottsdale, AZ; Mayo Clinic, Jacksonville, FL.

**Background:** Unstained recuts from formalin-fixed paraffin-embedded tissues are commonly collected for cooperative group studies. There is concern among pathologists that improper storage conditions can lead to antigen degradation. In an effort to quantify this effect, we compared the expression of HER1 and HER2 on two sets of identical cohort tissue microarrays (TMAs) from the N9831 HER2+ adjuvant phase III trial (NCT00005970; www.clinicaltrials.gov); one freshly cut set (cut April 18, 2011) and a second set stored at 4 degrees for over two years (cut between Nov, 2007 and Jan, 2008).

**Methods:** The two sets of TMA slides containing 1580 tumor samples from the N9831 cohort were treated identically using the AQUA method of quantitative immunofluorescence. HER1 was tested with D38B1 (rabbit monoclonal, Cell Signaling Technology, Inc.) and HER2 with CB11 (mouse monoclonal, Biocare, Inc.) on tumors from 695 patients (712 specimens) in the fresh TMAs and 779 patients (800 specimens) in the old TMAs in up to three-fold redundancy per specimen.

**Results:** Frequency distributions of the expression of HER2 revealed bimodality in the fresh TMAs compared to an attenuated distribution of the old cases. The average score of the entire cohort was significantly lower in old TMAs compared to fresh cuts (paired t-test, p<0.0001). Linear regression of the average HER2 scores from new TMAs versus the average scores from old TMAs showed a slope term of 0.52, which is statistically significantly different from the hypothetical value of 1 (p<0.0001). Regressions between any two fresh slides showed slopes close to 1.0. Similar results were seen for HER1, but fewer positive cases made the changes less dramatic.

**Conclusions:** The storage condition of tissue slides is a critical pre-analytical variable that can dramatically lower the score of HER1 and HER2, artificially. Thus, studies done on inadequately stored slides, either whole sections or TMAs, must be interpreted with caution. Tissue collection and analysis of biomarkers for cooperative group studies should not include unstained recuts, but rather, entire blocks or large cores from tissue blocks.

### PD05-06
**Determination of HER2 Status with Analysis of Plasma DNA by Digital PCR in Patients with Metastatic Breast Cancer.**
Graser EA, Gevensoelen H, Smith IE, Ashworth A, Turner NC. Institute of Cancer Research, London, United Kingdom; Royal Marsden Hospital, London, United Kingdom.

**Background:** A small proportion of originally HER2 negative primary breast cancers relapse with HER2 amplified disease. Identification of these cancers is important as these patients may benefit from HER2 targeting therapies. A proportion of free plasma DNA originates from the cancer, and accurate determination of HER2 copy number in plasma DNA has the potential to non-invasively determine HER2 status in patients with metastatic breast cancer. Digital PCR can differentiate small increases in DNA concentration with a much higher degree of accuracy than conventional quantitative PCR, and here we assess the potential of plasma DNA digital PCR to determine HER2 status.

**Methods:** We examined a cohort of 44 patients with metastatic breast cancer treated at the Royal Marsden Hospital who had received a median of 1.5 prior courses of chemotherapy for recurrent disease; 34 patients had ER positive and 9 HER2 positive (all 3+ positive HercepTest) disease as determined on biopsy of recurrent disease. Following informed consent, plasma samples were taken, free plasma DNA was extracted and concentration determined with LINE1 quantitative PCR. We designed a custom HER2 copy number TaqMan MBG-probe (Applied Biosystems) and two chromosome 17 peri-centromeric control probes TUFMP1 and UBBP4, avoiding known SNPs and regions of normal copy number variation. Digital PCR was performed in 384 well format on a 7900HT Fast RT-PCR system, with copy number ratio determined using the Poisson distribution.

**Results:** HER2 Digital PCR assay was initially tested on DNA from 9 HER2 amplified cell lines. HER2/UPPB4 ratio was increased in 9/9 HER2 amplified cell lines, and HER2/TUFMP1 in 7/9 due to co-amplification of the 17q peri-centromeric DNA in 2 cell lines. Dilution

<table>
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<th>Disease Free Survival</th>
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<td>72</td>
<td>0.70</td>
<td>0.40-1.21</td>
<td>0.36</td>
<td>87.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>High</td>
<td>115</td>
<td>27</td>
<td>0.59</td>
<td>0.30-1.01</td>
<td>0.05</td>
<td>85.1</td>
</tr>
</tbody>
</table>

Discordance between primary and metastatic tumors can impact significantly on patient management and suitability for HER2 targeted therapies in the setting of recurrent distant disease.

**Methods:** A literature search of the PubMed database was performed to identify all primary studies comparing HER2 status in matched primary and distant metastatic tumors between January 2005 and February 2011. Review and data extraction was performed by two independent reviewers. Eligible studies evaluated HER2 status by current ASCO guidelines. Studies comparing paired primary and axillary lymph nodes or synchronous primaries and metastases were excluded. Weighted pooled estimates were calculated.

**Results:** The original search strategy retrieved 1703 studies for which there were 62 eligible studies for full evaluation. Metastatic sites included bone, brain, lung and liver. Traztuzumab was received in three studies. Weighted summary estimate for negative to positive discordance was 23% (95% CI, 16.0-28%). Positive to negative discordance had a weighted summary estimate of 4% (95% CI, 0.0-6%).

**Discussion:** Meta analysis of studies adhering to ASCO guidelines for HER2 status determination revealed a significant rate of change to HER2 positive metastatic disease in HER2 unamplified primary breast cancers. This data suggests that nearly one quarter of patients may be denied the benefit of HER2 targeted therapy if HER2 primary status alone is used a the sole measure in determining therapeutic options upon distant recurrence.
experiments with SKBR3 DNA in non-amplified DNA demonstrated sensitivity down to ~1% levels. We selected HER2/UBBP4 digital PCR for examination of patient samples. Plasma DNA Digital PCR HER2/UBBP4 ratios differed substantially between HER2 non-amplified (mean 1.047, SD 0.149, median 1.046, range 0.70-1.264), and HER2 amplified cancers (median 2.231, range 1.138-7.89, p<0.001). Digital PCR HER2 ratios had a high diagnostic accuracy with ROC curve AUC 0.967 (95% CI 0.955-0.979). With a threshold Digital PCR HER2 ratio of 1.3 the test had 100% specificity and 89% sensitivity, with negative predictive value 97%. The single miscarried HER2 amplified patient had a low tumour burden. Results of a test set using sequential probability ratio test analysis will be presented at the conference.

Conclusions: Plasma DNA Digital PCR accurately determined HER2 status in patients with metastatic breast cancer. Analyses of plasma DNA have the potential to replace biopsy sampling of metastatic disease to guided targeted therapy.

PD05-07
Prospective Validation and Characterization of HER2 Positive Circulating Tumor Cells in Patients with HER2 Negative Metastatic Breast Cancer.

Background: Circulating tumor cells (CTCs) with evidence of HER2 amplification can occur in patients (pts) with clinically HER2 negative metastatic breast cancer. While these findings potentially have profound implications for CTCs as a biomarker for treatment, prospective validation and characterization of this subgroup is necessary.

Methods: We created a prospective cohort of pts with metastatic breast cancer that were HER2 negative by IHC and/or FISH on all available primary and metastatic biopsies. Blood samples were collected at study entry and then again at ≥ 3 weeks if available. CTCs were enumerated by a modification of the Veridex CellSearch Profile kit. FISH was performed on each CTC sample and reported as positive if the HER2/CEP17 ratio was ≥ 2.0. Analyses are descriptive.

Results: 66 pts were consented for study and this report includes the 65 pts with detectable CTCs. Median number of CTCs was 226 (range 112 to > 3000). At initial testing, 23 pts (35%) had HER2 positive CTCs, median HER2/CEP17 ratio of 3.4. 50% (11 of 22) of the pts with lobular or ductal/lobular histology had HER2 amplified CTCs, compared to only 27% (10 of 36) of patients with ductal histology. Women with ER positive disease had HER2 positive CTCs in 40% of cases (20 of 49) compared to 19% of ER negative pts (3 of 16). To assess concordance of HER2 amplification of CTCs over time, in 83% (10 of 12) pts; the 2 women with discordant HER2 amplified patient had a low tumour burden. Results of a test set using sequential probability ratio test analysis will be presented at the conference.

Conclusions: Plasma DNA Digital PCR accurately determined HER2 status in patients with metastatic breast cancer. Analyses of plasma DNA have the potential to replace biopsy sampling of metastatic disease to guided targeted therapy.

PD06-01
Molecular Classification with 21 Gene Assay (OncoType DX®) Shows in 196,967 ER Positive Patients High Frequency of Low Recurrence Score [LRS] in Both Node Positive (N+) and Negative (N-) Breast Cancer (BrCa) Cohorts. Definition of Chemoresistance Based on LRS with Cost and Guideline Implications.

BACKGROUND
Key retrospective analyses have shown no benefit from adjuvant CT [CT] in ER+, N-ve and N+ve BrCa pts with LRS by OncoType DX assay. (For N-ve, hazards [RR] = 1.31, 95% C.I.: 0.46, 3.78 (Paik et.al., 2006); for N+ve, RR=1.01, 95% C.I.: 0.54, 1.93 (Albain et.al. 2009)). LRS (RS<18) can therefore be equated to CT resistance (CTRES).

OBJECTIVES
1. To determine the frequency of CTRES determined by OncoType DX in the Genomic Health database of ER+, N-ve and N+ve pts.
2. To estimate the net cost savings resulting from avoidance of CT for CTRES pts, based on universal OncoType DX testing in ER+ pts being considered for CT.

METHODOLOGY
STEP 1
OncoType DX results were analyzed according to nodal status in the 196,967 ER+ tumor samples for CT candidates submitted to Genomic Health between Jan 2007 – Apr 2011.

STEP 2
We then calculated the $ cost saved, result of Oncotype DX, assuming:
i. of new BrCa pts, 42% could be candidates Oncotype DX and CT (ER+, high risk N-ve; and all ER+ N+ve)
ii. either 50% or 100% CT avoidance – according to oncology practice - among LRS cases, estimating the CT cost/pt = $15,000 US; and OncoType DX cost/pt = $4,000 US.

RESULTS - STEP 1
LRS based on OncoType DX was more prevalent in N+ pts (chi sq p <0.0001)

<table>
<thead>
<tr>
<th>Category</th>
<th>N-</th>
<th>N+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low (LRS&lt;18)</td>
<td>99,259</td>
<td>8,170</td>
</tr>
<tr>
<td>Int (LRS 18-30)</td>
<td>61,898</td>
<td>5,419</td>
</tr>
<tr>
<td>High (LRS&gt;31)</td>
<td>21,867</td>
<td>833</td>
</tr>
<tr>
<td>TOTAL</td>
<td>183,024</td>
<td>12,522</td>
</tr>
</tbody>
</table>

RESULTS - STEP 2
CT Cost [in millions, USD] / 1,000 newly diagnosed cases who are CT and Oncotype DX candidates, of whom 50% [conservative estimates] will have LRS

<table>
<thead>
<tr>
<th>Category</th>
<th>N-</th>
<th>N+</th>
</tr>
</thead>
<tbody>
<tr>
<td>CT</td>
<td>18</td>
<td>11.25</td>
</tr>
<tr>
<td>CT gene assay</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Health system cost</td>
<td>18</td>
<td>11.25</td>
</tr>
<tr>
<td>Cost Savings</td>
<td>0</td>
<td>7.5</td>
</tr>
<tr>
<td>Total cost savings / year / U.S.</td>
<td>$330.75 mil</td>
<td></td>
</tr>
</tbody>
</table>

Thus, in USA, with 225,000 new BrCa pts / year, and 94 500 [42%] candidates for Oncotype DX, over 47,250 pts will have LRS, with overall savings of $330.8 mil /year with 100% CT avoidance. In Canada, with 25,000 estimated new BrCa pts/year, corresponding savings will be $46.2 mil /year.

CONCLUSIONS:
1. Low RS status, presently the most reliable CTRES biomarker, is expressed with higher frequency among N+ve (59%) vs N-ve (54%) ER+ breast cancer pts.
2. If all eligible cases had the Oncotype DX test, and all with LRS would avoid CT, close to 50,000 pts in USA, and over 5,200 in Canada will be spared ineffective CT and associated toxicity / year, with hundreds of million dollars saved each year.
Conclusions: Reallocation of adjuvant chemotherapy based on Oncotype DX test results was associated with improvements in survival and quality-adjusted life expectancy in this modeling analysis. At a willingness to pay threshold of GBP 20,000 per QALY (commonly quoted as representing good value for money in the UK), probabilistic sensitivity analysis showed that there was a 99.6% probability that Oncotype DX would be cost-effective versus current clinical practice.

PD06-03
Cost Effectiveness Analysis of BRCA1/2 Genetic Testing.
Li Q, Holland M, Huston A, Noyes K. University of Rochester School of Medicine, Rochester, NY

Background: Family history of breast and/or ovarian cancer is associated with an increased risk to carry a BRCA1 or BRCA2 gene mutations which significantly increase a woman’s risk to develop breast and/or ovarian cancer. This study examines whether BRCA1/2 genetic testing (intervention) remains cost-effective compared to no genetic testing strategy (control) from the societal and private payer perspectives given updates in healthcare practice, policy and clinical specificity of testing in the last 10 years.

Data and method: We updated a previous published semi-Markov model (Holland et al., Value in Health, 2009. 12(2): p. 207) to include new information about associated costs and treatment strategies and test specificity. The target population is 35 year old asymptomatic US women with an elevated risk of BRCA1/2 genetic mutation. The estimates of probability (prevalence, risk, preference and mortality), cost, and utility are derived from published reports and BRCA1/2 test technical documentation. We conducted the basecase cost-utility analysis and a series of sensitivity analyses by varying pretest probability of BRCA1/2 mutation, clinical sensitivity of BRCA1/2 testing, initial utility after BC diagnosis and patient preference in the first year after mastectomy, cost of BRCA1/2 testing, and out-of-pocket costs.

Results: From the societal perspective, the “no test” strategy was estimated to cost $162K and resulted in 20.2 QALY gain over a patient’s lifetime. The “test” strategy was estimated to cost $172K and result in 20.5 QALY gain, resulting in the incremental cost-effectiveness ratio (ICER) of $30.6K/QALY. From a private payer perspective, the ICER was $36.8K/QALY. By conducting sensitivity analyses, we concluded that the model was robust to variation in the model parameters. Within the ranges of most variable estimates, the test strategy was more cost effective compared to the no-test strategy. The initial utility after BC diagnosis (basecase value 0.75) does not impact the choice of preferred strategy (testing is always preferred). Based on the probability of mutation for women with family history (basecase value 8.7%), testing is preferred if probability is greater than 3.1%, while no testing is preferred for values <3.1%. Only if cost of genetic testing is greater than $8,948 testing would it no longer be cost effective, which is however far beyond even the upper bound of cost estimate ($4,500). As long as the sensitivity of BRCA testing remains greater than 80% and initial utility after mastectomy <0.9, the testing is preferred over no testing.

Discussion: The strategy of BRCA1/2 testing of women at high risk for BRCA1/2 mutations and treatment of BRCA1/2 positive women is cost-effective compared to “no genetic testing” strategy. Cost of the actual test is not a barrier to its cost-effectiveness. Despite adding MRI to breast cancer surveillance in high risk population and increases in healthcare costs, BRCA1/2 testing remains cost-effective from both the societal and from a private payer perspectives for an unaffected population with BRCA1/2 prevalence of greater than 3%.

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### Table: Cost-Effectiveness Analysis of BRCA1/2 Genetic Testing

<table>
<thead>
<tr>
<th>Scenario</th>
<th>Life expectancy (years)</th>
<th>Rate of BRCA1/2 mutations</th>
<th>Cost of BRCA1/2 testing (GBP)</th>
<th>Quality-adjusted life expectancy (QALYs)</th>
<th>Cost/QALY gained (GBP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basecase</td>
<td>11.39</td>
<td>8.7%</td>
<td>4,500</td>
<td>11.54</td>
<td>0.14</td>
</tr>
<tr>
<td>Sensitivity analysis</td>
<td>12.754±3.91</td>
<td>8.7%</td>
<td>4,500</td>
<td>11.54</td>
<td>0.14</td>
</tr>
</tbody>
</table>

Values shown are per patient.

---

### Table: Long-term clinical and cost outcomes with Oncotype DX testing versus current clinical practice

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Current clinical practice</th>
<th>Oncotype DX testing</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Life expectancy (years)</td>
<td>11.39</td>
<td>12.754±3.91</td>
<td>0.16</td>
</tr>
<tr>
<td>Quality-adjusted life expectancy (QALYs)</td>
<td>11.39</td>
<td>11.54</td>
<td>0.14</td>
</tr>
<tr>
<td>Cost (GBP)</td>
<td>31,847±24</td>
<td>12,754±3.91</td>
<td>887±69</td>
</tr>
</tbody>
</table>

Values shown are per patient.

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**PD06-02**
Cost-Effectiveness Evaluation of the Oncotype DX® Breast Cancer Assay in Clinical Practice in the UK.

Holt SDH, Bennett H, Bertelli G, Valentine WJ, Phillips CJ. Prince Philip Hospital, Llanelli, United Kingdom; Cardiff Research Consortium, Cardiff, United Kingdom; Singleton Hospital, Swansea, United Kingdom; Ossian Health Economics and Communications, Basel, Switzerland; Swansea University, Swansea, United Kingdom

Objective: Optimizing therapeutic regimens for breast cancer patients has an important role to play in improving outcomes and planning the best use of National Health Service resources. Oncotype DX® testing has been shown to provide clinically valuable information in addition to traditional measurements (such as tumor size, tumor grade and lymph node status) to support chemotherapy treatment decision making in women with estrogen receptor positive (ER+), node-negative and up to 3 node-positive breast cancer. The aim of this study was to evaluate the cost-effectiveness of Oncotype DX testing to support adjuvant therapy decision making versus current clinical practice in the treatment of patients with ER+, early-stage breast cancer in the UK.

Methods: A Markov model was developed to make long-term projections of distant recurrence, survival, quality-adjusted life expectancy and direct medical costs for patients with ER+, node-negative or micrometastatic (pN1mic) early-stage breast cancer. Scenarios using conventional diagnostic procedures (including Adjuvant! Online and the Nottingham Prognostic Index) or Oncotype DX testing to inform treatment recommendations for adjuvant therapy were modeled. The model relied on data from an ongoing study in Wales for treatment recommendations (with and without Oncotype DX), landmark Oncotype DX studies for the risk of recurrence, and UK-specific life tables for mortality. Costs were derived from published UK sources and expressed in 2010 Pounds Sterling (GBP). Future costs and clinical benefits were discounted at 3.5% annually. Probabilistic and deterministic sensitivity analyses were performed.

Results: Oncotype DX was projected to increase mean life expectancy by 0.16 years and mean quality adjusted life expectancy by 0.14 years compared to current clinical practice over a 30-year time horizon (see table). Clinical benefits were driven by optimized allocation of adjuvant chemotherapy in the Oncotype DX group. Direct medical costs were estimated to be higher with Oncotype DX testing, leading to an incremental cost-effectiveness ratio (ICER) of approximately GBP 6,232 per QALY gained for Oncotype DX versus current clinical practice in the UK. Sensitivity analysis showed that the cost-effectiveness of Oncotype DX testing was most sensitive to variations in patient age and net changes in chemotherapy for low risk patients.

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**Long-term clinical and cost outcomes with Oncotype DX testing versus current clinical practice**

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Current clinical practice</th>
<th>Oncotype DX testing</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Life expectancy (years)</td>
<td>11.39</td>
<td>12.754±3.91</td>
<td>0.16</td>
</tr>
<tr>
<td>Quality-adjusted life expectancy (QALYs)</td>
<td>11.39</td>
<td>11.54</td>
<td>0.14</td>
</tr>
<tr>
<td>Cost (GBP)</td>
<td>31,847±24</td>
<td>12,754±3.91</td>
<td>887±69</td>
</tr>
</tbody>
</table>

Values shown are per patient.
**PD06-04**

**Uptake and Economic Impact of First-Cycle Colony Stimulating Factor Use during the Adjuvant Treatment of Breast Cancer.**

**Hershman DL, Wilde ET, Wright JD, Buono DL, Kalinsky K, Malin J, Tsai WY, Neugut AI. Columbia University, New York, NY; Mailman School of Public Health, New York, NY; Greater Los Angeles VA Healthcare System, Los Angeles, CA**

**Background:** In 2002, pegfilgrastim was approved by the FDA and the benefits of dose-dense breast cancer (BC) chemotherapy were reported. A meta-analysis revealed these benefits were limited to hormone receptor negative tumors (20% risk reduction), where hormone receptor positive cancers had a minimal 2% risk reduction. We examined first-cycle growth factor use (FC-CSF) before and after 2002 and estimated US expenditures for dose-dense chemotherapy by hormone receptor status.

**Methods:** We identified subjects in SEER-Medicare >65 years old with stage I-III BC between 1998 and 2005 who had ≥1 chemotherapy claim within one year of diagnosis. We classified those with an average cycle length <21 days as having had dose-dense therapy. The associations of patient, tumor, and physician-related factors with receipt of any CSF and FC-CSF use were analyzed using GEE. Costs and event-free life-years saved were estimated for patients by HR status.

**Results:** Among the 11,143 patients identified, 5,356 (48.1%) had CSF claim during therapy and 2,095 (18.8%) had FC-CSF. CSF use increased from 25.4% to 70.9%, FC-CSF increased from 6.4% to 46.9% and pegfilgrastim increased from 5% to 85%. During this time receipt of the combination of an anthracycline and a taxane increased from 12% to 47%. In a multivariable analysis, any CSF use was associated with age, treatment by an oncologist, and chemotherapy type; and negatively associated with black/Hispanic race, rural residence and shorter chemotherapy duration. FC-CSF use was associated also with higher SES. For hormone receptor negative patients, the cost per event-free life-year saved was estimated for patients by HR status.

**Conclusions:** Our study demonstrated a widespread increase in the use of CSF’s and specifically first cycle pegfilgrastim in elderly women receiving adjuvant chemotherapy for breast cancer. This 4-fold increase came shortly after published reports of the benefits of dose-dense therapy. While this approach is very cost-effective in women receiving adjuvant chemotherapy for breast cancer. This approach is very cost-effective in women receiving adjuvant chemotherapy for breast cancer. This approach is very cost-effective in women receiving adjuvant chemotherapy for breast cancer. This approach is very cost-effective in women receiving adjuvant chemotherapy for breast cancer.

**PD06-05**

**Primary and Secondary Pegfilgrastim Utilization in Adjuvant Chemotherapy for Breast Cancer in the Community.**

**Patt DA, Espirito JL, Turnwald B, Hoverman JR, Neubauer MA, Busby LT, Brooks BD, Kołodziej MA, Anderson RW, Beveridge RA. On Behalf of US Oncology Pathways Task Force and Clinical Content Development Team. Texas Oncology, TX; US Oncology, TX; Kansas City Cancer Center, KS; Rocky Mountain Cancer Center, CO; New York Oncology Hematology, NY**

**Background**

Various factors are taken into consideration in the selection of adjuvant breast cancer (BC) chemotherapy (CT) regimens for patients. Choice of CT, schedule, duration, and supportive care affects costs and toxicity. Understanding clinical practice utilization patterns are important when making cost estimates of adjuvant therapy. Because pegfilgrastim is a large driver of cost it is important to understand the utilization characteristics. We aimed to characterize primary and secondary pegfilgrastim use during neoadjuvant/adjuvant (N/Ad) chemotherapy by regimen type. While initial data suggests the incidence of febrile neutropenia (FN) is low among some docetaxel containing regimens, we wanted to further characterize pegfilgrastim utilization, as previous utilization studies suggested it was higher than expected.

**Methods**

Using the US Oncology iKnowMed™ EHR database, we retrospectively identified female BC patients (pts) diagnosed with stage I-III BC, between 7/2006 and 11/2010. Secondary diagnoses were excluded. Pts were characterized by age, ER and HER2 status, tumor size, grade, and nodes. CT utilization was determined by the number of pts assigned an N/Ad line of therapy (LOT) during the study period. Regimens were categorized by CT title and drugs. Clinical trial pts were included. Pegfilgrastim utilization was characterized if administered within 6 months of being assigned to an N/Ad CT regimen, and was captured as primary prophylaxis if the first dose was administered ≤5 days of C1D1 of a regimen, and secondary prophylaxis if >5 days.

**Results**

General chemotherapy and pegfilgrastim utilization characteristics were previously reported. This report captures primary vs. secondary pegfilgrastim use. During the time period, 40,881 BC pts were identified. Of these, 15,328 pts (37%) were assigned an N/Ad CT regimen and 72% (11,022 pts) received pegfilgrastim at any time within 6 months of their N/Ad regimen. Docetaxel containing regimens (TC, TAC, TCH) and dose-dense regimens accounted for the majority of all pegfilgrastim use. Pegfilgrastim utilization with the TC regimen was 70%, and represented 25% of all N/Ad pegfilgrastim utilization. The vast majority of utilization for TC and TCH was primary prophylaxis as detailed below:

<table>
<thead>
<tr>
<th>Regimen</th>
<th>Primary utilization % of total</th>
<th>Secondary utilization % of total</th>
<th>None % of total</th>
<th>% of total</th>
<th>Grand Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC</td>
<td>2089 53%</td>
<td>637 16%</td>
<td>1183 30%</td>
<td>3909</td>
<td></td>
</tr>
<tr>
<td>TCH</td>
<td>795 53%</td>
<td>302 20%</td>
<td>398 22%</td>
<td>1495</td>
<td></td>
</tr>
</tbody>
</table>

**Conclusions**

While primary prophylaxis in regimens like dose-dense AC and TAC are expected, the primary utilization of pegfilgrastim in TC and TCH is higher than expected based on published clinical trial experience. The incidence of FN has been reported at 5% in the clinical trial by Jones et al with TC, however subsequent reports suggest the incidence of FN may be higher than expected. Our results demonstrate high primary prophylaxis utilization adoption in clinical practice. With the availability of generic docetaxel, commonly used drugs in adjuvant BC except trastuzumab have generic equivalents. Pegfilgrastim will be the largest cost driver in women receiving adjuvant chemotherapy.
and should be considered among cost estimates. This study may underestimate utilization of pegfilgrastim if it was administered outside of the cancer center.

**PD06-06**

**Is Adjuvant Trastuzumab Economically Justified in Her-2/neu Positive T1bNO Breast Cancer?**

**Skedgel C, Rayson D, Younis T, Capital Health, Halifax, NS, Canada**

**Background:** A number of clinical trials and cost-utility analyses of adjuvant trastuzumab (aTZ) for Her-2/neu positive breast cancer suggest favourable efficacy and safety along with acceptable cost-effectiveness. These evaluations, however, were based on patient cohorts with moderate-to-high risk disease, including node positive or high risk node negative disease with tumor sizes greater than 1 cm (≥T1cN0). The role of aTZ for patients with lower risk disease, such as T1bN0, remains unclear in light of the varying 10-year relapse risks (10-30%) reported to date.

**Objective:** To estimate the cost-utility of chemotherapy plus aTZ versus chemotherapy alone in Her-2/neu positive breast cancer patients with lower recurrence risk in terms of cost per quality-adjusted life year (QALY) gained.

**Methods:** A state-transition economic model was developed to estimate the incremental costs and quality-adjusted life year (QALY) gains associated with a strategy of chemotherapy plus aTZ relative to chemotherapy alone over a 25-year analysis horizon. The model consisted of four broad health states, stratified with or without cardiotoxicity: 1) disease-free, 2) local recurrence, 3) distant recurrence and 4) death. Given the variability in reported risk estimates for T1bN0 disease, a range of 10-year baseline recurrence risks (10-30%) in the absence of chemotherapy or aTZ was examined. The clinical benefit of chemotherapy was assumed to differ according to age (<50 vs. ≥50 years). The hazard ratio of recurrence with aTZ (HR=0.64) and the rate of associated adverse cardiac events were derived primarily from the HERA clinical trial. Utility weights and the costs of local and distant cancer recurrence were derived from the literature, while the costs of adjuvant and palliative TZ were derived from our previous work. The model took a direct payer perspective, with costs reported in 2011 Canadian dollars (CDN$). Costs and QALYs were both discounted by 3% annually. The reference analysis assumed 3 years of clinical benefit from chemotherapy and aTZ. A series of one-way sensitivity analyses tested the impact of longer benefit as well as the impacts of measuring outcomes in terms life years rather than QALYs, a lifetime analysis horizon and different HRs.

**Results:** The reference analysis observed that the cost per QALY gained was greater than CDN$100,000 across the entire range of recurrence risks tested. One-way sensitivity analyses observed that considering life years rather than QALYs, a lifetime analysis horizon, extending the duration of benefit to 5 years or improving the HR to 0.54 each little to improve the overall economic favorability of aTZ, even at the higher range of recurrence risk.

**Conclusions:** The cost-utility of adjuvant trastuzumab appears unfavourable in Her-2/neu positive breast cancers with a baseline 10-year relapse risk of less than 30% without treatments, such as in T1bN0 disease. Specific estimates of cost-utility await more precise estimates of the recurrence risk in patients with T1bN0 disease.

**PD06-07**

**COMPliance and Arthralgias in Clinical Therapy (COMPACT): Assessment of the Incidence and Severity of Arthralgia, Treatment Costs and Compliance within the First Year of Adjuvant Anastrozole Therapy.**

**Hadji P, Blettner M, Bolten WW, Harbeck N, Hindenburg H-J, Jackisch C, König K, Lueck H-R, Rief W, Zaan S, Klein P, Kreienberg R. Philipps-University, Marburg, Germany; Institute for Medical Biometry and Epidemiology, Mainz, Germany; Klaus-Miehlke-Klinik for Rheumatology, Wiesbaden, Germany; Breast Center, Women’s University Hospital, Cologne, Germany; Berufsverband Niedergelassener Gynäkologischer Onkologen e.V., Berlin, Germany; Klinikum Offenbach, Germany; Berufsverband der Frauenärzte e.V., Germany; Gyn. Oncological Practice, Hannover, Germany; AstraZene, Germany; DSH Statistics, Germany; University Women’s Hospital, Ulm, Germany**

**Introduction:** Aromatase inhibitors (AI) are well established as adjuvant endocrine treatment for postmenopausal (PMP) women with HR+ early breast cancer (EBC). However, according to retrospective data, compliance to adjuvant endocrine therapy for EBC may drop to below 70% after one year and to as low as 50% by year 4. In clinical trials, AI are significantly more frequently associated with arthralgia than tamoxifen. Yet, prospective real world data on the effects of AI-associated arthralgia on patient compliance, patient outcomes as well as treatment costs of arthralgia are lacking.

**Methods:** COMPACT is an open, prospective, non-interventional study assessing the incidence and severity of arthralgia, treatment costs, and compliance within the first year of adjuvant anastrozole therapy in PMP women with HR+ EBC. The study is sponsored by AstraZeneca Germany and supported by three major German health insurance funds [GWQ ServicePlus AG, DAK, TK]. Patients on adjuvant treatment for 3–6 months were enrolled at 620 breast centres and practices throughout Germany and stratified by, a) initial adjuvant anastrozole therapy or, b) switch from tamoxifen to anastrozole. All patients receive regular standardized information about EBC from baseline to week 20 to support treatment compliance. Data on patient demographics, occurrence of and treatment of arthralgia, and quality of life will be collected at baseline, 3, 6 and 9 months. Primary endpoints are scaled data on arthralgia, assessed with a visual analogous scale (VAS) via patient questionnaire, and compliance to anastrozole in both strata, assessed by patient and investigator questionnaire. Secondary endpoints include the incidence of arthralgia, treatment costs, reasons for non-compliance, and the influence of arthralgia on clinical outcome. For a subgroup of patients data on arthralgia treatment and compliance will be validated with corresponding data of the participating health insurance funds.

**Results:** Between April 2009 and February 2011, 2313 patients were recruited, 2007 receiving upfront anastrozole and 306 patients on switch therapy. Preliminary baseline data for 2313 patients show the following patient characteristics: mean age 64.5 years, mean BMI 27.7. Only 16.8% of patients had received hormone replacement therapy prior to their cancer. 41.5 % of patients had concomitant symptoms relating to skeleton or musculature, and 11.9% stated arthralgias existing prior to anastrozole treatment. 13.1% reported a worsening of pre-existing arthralgias or new arthralgia after starting on anastrozole treatment.

**Conclusion:** COMPACT aims to provide valid real world data on the incidence and severity of AI-associated arthralgia, treatment modalities and treatment costs. Our results will help to understand and better counsel patients about AI-associated arthralgia to improve adherence to AI-treatment, breast cancer outcomes, and therapy costs.
**PD06-08**  
Projecting the Impact of Adopting Trial Results  
Esserman LJ, Mohan AJ, Park C, Berry DA, Ozanne E, Alvarado MD. UCSF; M.D. Anderson  

**Background:** There is often a substantial delay between the presentation of clinical trial results and change in practice. Reasons for slow adoption include the disruption of practice routines, doubt about the validity of results, financial disincentive, and resistance to change. We propose a decision-making process to inform adoption that considers three factors: likelihood the trial's conclusions will not change with longer follow up, and consequences of early and late adoption. We apply this framework to the published 4-year results of the international TARGIT-A trial, which compares intraoperative radiation therapy (IORT) to external beam radiation therapy (EBRT).

**Methods:** To find whether the trial results will remain robust, we reviewed the TARGIT-A trial's annual hazards for local recurrence (LR). We then reviewed 5-10 year LR rates from recent clinical trials with similar patient populations undergoing RT, no RT or partial breast RT. To assess the impact of an early change in practice, a Markov model was used to evaluate life expectancy, quality adjusted life years (QALYs), and cost. Sensitivity analysis estimated the impact of varying the LR rate 1-20x the TARGIT-A 4 year results. To estimate the impact of late adoption, we generated the expected number of N0, grades 1 & 2, ductal cancers in postmenopausal women (>50 yrs) from SEER and US Census Bureau data. Using Medicare rates, costs of EBRT vs. IORT were compared and potential savings to the health care system calculated.

**Results:** The TARGIT-A peak hazard for LR for IORT and EBRT occurred at 3 years. In the START and ATAC trials, the peak local recurrence Kaplan Meier estimates are between 2 and 3 years. In other trials (ATAC, and in the 2005 RT Lancet overview) there is no second peak of recurrence, making it unlikely that longer follow up will change the conclusion of the TARGIT-A results. The LRR in similar trials have dropped steadily over time for post menopausal, stage I patients with or without RT. Impact of early adoption: If treatment were adopted early, the impact on life expectancy and QALYs would be minimal unless IORT LR rate exceeds 20% over a 10 year period of time. Impact of late adoption: In the US, 45% of new breast cancer cases fit the described population. EBRT costs $6,400 more than IORT per patient on average. If adoption were delayed 5 years to allow the trial results to mature, 70,136 patients per year are expected to receive EBRT resulting in a societal burden of >$2.2 billion barring the capital investment required for new technology. This also assumes that patients have equal utility for a single dose of IORT as they do for 3-6 weeks of postoperative radiation therapy. If IORT utility is higher than that of EBRT, then the IORT strategy is both more effective and less costly.

**Conclusion:** The process of modeling the impact of both early and late adoption when considering the stability of trial results can serve as a tool to evaluate whether to change practice. This analysis was tested on the results of the TARGIT-A trial, and demonstrated that prompt adoption of the IORT intervention would cause minimal harm, provide an improved quality of life, and offer significant societal savings.

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**PD06-09**  
Early Predictors of Prolonged Unemployment after a Diagnosis of Breast Cancer.  
Blinder V, Patil S, Eberle C, Maly RC. Memorial Sloan-Kettering Cancer Center, New York, NY; University of California, Los Angeles, CA  

**Introduction:** Low-income women may be more vulnerable to job loss and prolonged work absence after a diagnosis of breast cancer than their higher-income counterparts. This can have important adverse financial consequences on breast cancer survivors. The identification of early risk factors for long-term unemployment could inform interventions to help patients avoid this outcome.

**Methods:** A consecutive sample of 921 low-income, underinsured or uninsured, English and/or Spanish-speaking women treated for breast cancer through the California Breast and Cervical Cancer Treatment Program was recruited and surveyed 6 months after a diagnosis of breast cancer. Participants completed follow-up telephone surveys at 18, 36, and 60 months post-diagnosis; 539 remained in the cohort at 60 months. This analysis includes only the 315 (58%) women who were employed before diagnosis. Our primary study outcome was prolonged unemployment, defined as being unemployed at every survey. Baseline characteristics (measured in the 6-month survey) were compared between women with prolonged unemployment and those who were working 60 months after diagnosis. Results: The median age of the study sample was 50, and 98% had a baseline annual household income <$40,000. Eighty-five of 315 (27%) women who were employed before diagnosis had prolonged unemployment after diagnosis. In contrast, 168 (53%) were working at 60 months. Baseline predictors of prolonged unemployment after diagnosis include lower household income (p=0.003), inadequacy of financial resources to cover needs (40% vs. 23%, for inadequate vs. adequate, p=0.006), lower education (43%, 30%, and 23% among those who did not complete high school, had a high school diploma, and had a college diploma, respectively, p=0.04), higher comorbidity burden (p=0.006), higher cancer stage at diagnosis (p=0.001), and receipt of chemotherapy (p=0.008). Variables found not to be associated with prolonged unemployment include age, ethnicity, acculturation, marital status, children in the home, social support, job type, type of breast surgery, type of axillary surgery, endocrine therapy, and radiation therapy. In a multivariable analysis that included ethnicity, education, income, chemotherapy, and comorbidity, the latter three variables remained statistically significant predictors of prolonged unemployment, but education and ethnicity were not significantly associated with the outcome. Discussion: More than a quarter of women treated for breast cancer never returned to work during the five years after their diagnosis. In this low-income sample, those with the lowest income appeared to be especially vulnerable to prolonged unemployment, even when controlling for education. It is possible that the income provided by a low-paying job provides a marginal benefit, if any, over disability income, and additional research is needed to better understand this relationship and its financial and psychosocial consequences. Clinical variables also were important predictors of prolonged unemployment. Women with a higher comorbidity burden and those treated with chemotherapy appear to be most vulnerable to prolonged unemployment. This may have clinical implications for informed decision-making between oncologists and their patients.
PD06-10
Breast Cancer Patient Distress Associated with Difficulties Navigating the Costs Associated with Care: Results from a National Education Program.

Compounding the stressors related with a diagnosis of breast cancer can be the associated direct and indirect costs of cancer care. To address this need, the Cancer Support Community (CSC) in 2009 developed an evidence-based, professionally-led national education workshop for cancer patients and caregivers entitled Frankly Speaking About Cancer: Coping with the Cost of Care. As part of this workshop, participants completed a survey describing their experiences coping with the cost of cancer care. To date, responses from 465 participants (representing 46 workshops) have been analyzed. Of those participants, 105 are women diagnosed with breast cancer. Though workshop participants were affected by a wide variety of cancer diagnoses, half of those with cancer attending the workshop (50%) were affected by breast cancer. No significant differences between breast cancer patients and individuals with other cancers were found. Analyses are based on responses from breast cancer patients only. Participants’ pre-workshop rating of their understanding about the financial aspects of their breast cancer care was low (m = 2.8, s.d. = 1.0) and was significantly less than their level of knowledge post-workshop (m = 4.1, s.d. = 0.7, p < .05).

Most attendees (72.8%) reported experiencing some degree of emotional distress from trying to manage cancer care costs, and nearly one-third of attendees (30.1%) reported significant distress. Most (64.8%) reported that their healthcare team did not discuss financial aspects of care with them. Of attendees whose team did discuss it with them, typically it was a social worker, physician, or nurse. Of those who had this discussion, only 34% reported that this information was actually useful to them. Not surprisingly then, attendees reported they had looked elsewhere for information about managing the costs of care, such as patient support organizations (40.2%), the Internet (43.5%), and other patients (41.3%).

A positive to arise from the workshop is that most participants (69.9%) reported the intention to discuss financial aspects of their care with their healthcare team based on what they had learned from the workshop. Intention to have this discussion with their healthcare team was both positively correlated with having experienced emotional distress about the cost of their care (r = .29, p < .05) as well as negatively correlated with their level of pre-workshop knowledge about financial issues in breast cancer care (r = -.28, p < .05). Taken together, these data highlight significant obstacles that individuals face in receiving meaningful information relevant to managing the costs associated with cancer care.

PD07-01
Sequential Treatment with Epirubicin/Cyclophosphamide, Followed by Docetaxel vs. FEC120 in the Adjuvant Treatment of Breast Cancer Patients with Extensive Lymph Node Involvement: Final Survival Analysis of the German ADEBAR Phase III Study.
Janni WJ, Harbeck N, Sommer H, Rack B, Salmen J, Augustin D, Simon W, Jueckstock J, Wischnik A, Anneke K, Melcher C, Friese K, Kiechle M, Heinrich-Heine-University, Duesseldorf, Germany; University Cologne, Cologne, Germany; Ludwig-Maximilians-University, Munich, Germany; Mammazentrum Ostbayern, Deggendorf, Germany; Robert-Bosch-Krankenhaus, Stuttgart, Germany; Zentalklinikum, Augsburg, Germany; Technische Universität, Munich, Germany

Background: Based on meta-analytic evidence, taxane containing adjuvant chemotherapy has been established as standard treatment in node-positive breast cancer. However, in the MA-21 study, adriamycin-cyclophosphamide, followed by paclitaxel (AC-P) was significantly inferior to the gold standard of anthracycline treatment, FEC120 (Burnell, SABCS 2006). We prospectively compared a sequential epirubicin-docetaxel chemotherapy regimen to FEC120.

Patients and Methods:
The ADEBAR study was a multicenter phase III trial (n=1502) to evaluate whether breast cancer (BC) pts with > 3 axillary lymph node metastases benefit from a sequential anthracycline-docetaxel regimen (E90C–D: 4 cycles epirubicin [E] 90 mg/m² plus cyclophosphamide [C] 600 mg/m² q21 days followed by 4 cycles docetaxel [D] 100mg/m² q21 days) compared to dose-intensive anthracycline-containing polychemotherapy (FE120C: 6 cycles E 60 mg/m² d 1+8, 5-FU 500mg/m² d 1+8 and C 75 mg/m² d 1-14, q4 weeks). The median follow-up time will be 60 months.

Results:
Treatment was stopped prematurely in 3.7% of the pts in the E90C–D arm and in 8.0% in the FE120C arm due to toxicity (p=0.0009). Antibiotic treatment was given in 10.4% (E90C–D) vs. 19.7% (FE120C), G-CSF support in 39.2% vs. 61.4%, respectively (p=0.0001). Haematological toxicity (leucopenia, neutropenic fever, thrombocytopenia, anemia) was significantly higher in the FE120C-arm. Mature final 5-year-survival data will be presented at the SABCS meeting 2011.

Conclusion:
Different toxicity profiles given, hematological toxicity in the FE120C group was more severe than in the E90C–D. Maturity survival data will be discussed in this context.

PD07-02
Docetaxel Is Superior to Paclitaxel Given Every Three Weeks in Post Operative Patients with Node-Positive Breast Cancer: Results of the Final Analyses of the NSAS-BC (National Surgical Adjuvant Study of Breast Cancer) 02 Trial from Japan.
Watanabe T, Kuranami M, Inoue K, Masuda N, Aogi K, Ohno S, Iwata H, Mukai H, Uemura Y, Ohashi Y. Hamamatsu Oncology Center, Hamamatsu, Japan; Kitasato University Hospital, Sagamihara, Japan; Saitama Cancer Center; Inachou-Okumo, Japan; NHO Osaka National Hospital, Osaka, Japan; Shikoku Cancer Center, Matsuyama, Japan; Kyushu Cancer Center, Fukuoka, Japan; Aichi Cancer Center, Nagoya, Japan; National Cancer Center, Kashiwa, Japan; University of Tokyo, Tokyo, Japan

Background: Four cycles of doxorubicin plus cyclophosphamide (4-AC) followed by four cycles of a taxane is widely used for
postoperative chemotherapy in breast cancer (BC). Concern about relatively rare, but life-threatening toxicity of anthracyclines such as heart failure and secondary leukemia has promoted research to seek anthracycline-free regimens. Since 1990’s when taxanes were introduced, docetaxel (DTX) is used interchangeably with paclitaxel (PTX) for the treatment of BC, but they may differ more than initially anticipated. We conducted this trial to test two hypotheses: (1) Eight cycles of a taxane is not inferior to 4-AC followed by four cycles of a taxane; (2) one taxane is superior to the other.

**Methods:** Eligibility included a diagnosis of clinical stage I-IIIA and axillary node-positive BC, an age younger than 71 years and with performance status of 0 to 1. Patients were randomly assigned to receive either one of the following regimens; ACP: 4-AC (doxorubicin 60 mg/m² and cyclophosphamide 600 mg/m² q3w x 4) followed by 4-PTX (175 mg/m² q3w x 4)

ACA: 4-AC followed by 4-DTX (75 mg/m² q3w x 4)

8-PTX: PTX (175 mg/m² q3w x 8)

8-DTX: DTX (75 mg/m² q3w x 8)

Comparisons included PTX vs. DTX (ACP + 8-PTX vs. ACD + 8-DTX) and +AC vs. -AC (ACP + ACD vs. 8-PTX + 8-DTX). The primary endpoint was disease-free survival (DFS), and the secondary endpoints include overall survival (OS). The trial was powered to prove the non-inferiority of +AC to -AC (threshold hazard ratio 1.32) in terms of DFS. DFS was also compared between PTX and DTX to determine any superiority.

**Results:** A total of 1,060 eligible patients were accrued at 84 centers between December 2001 and April 2006. There were 348 DFS events between December 2001 and April 2006. There were 348 DFS events and 166 deaths after a median followup of 72.2 months. DTX was introduced, docetaxel (DTX) is used interchangeably with paclitaxel (PTX) for the treatment of BC, but they may differ more than initially anticipated. We conducted this trial to test two hypotheses: (1) Eight cycles of a taxane is not inferior to 4-AC followed by four cycles of a taxane; (2) one taxane is superior to the other.

**Nausea and vomiting** were more frequent with +AC than -AC. Edema and febrile neutropenia were more frequent with DTX than PTX. The incidence of sensory neuropathy was higher with PTX than DTX and it lasted for more than one year of the end of PTX treatment.

**Conclusions:** When AC, PTX and DTX were given every three weeks, both DFS and OS were better in the arms including DTX than in those including PTX. AC followed by a taxane can be replaced by 8-DTX.

**PD07-03**

**Phase II Trial of Adjuvant TC (Docetaxel/Cyclophosphamide) Plus Trastuzumab (HER TC) in HER2-Positive Early Stage Breast Cancer Patients.**

Jones S, Collea R, Paul D, Oritz R, Sedlacek S, Favret AM, Gore, Jr H, Lindenquest DL, Holmes EA, Allison MAK, Steinberg MS, Stokoe C, Portillo RM, Crockett M, Wang Y, Asmar L, Robert N, O’Shaughnessy J. US Oncology, The Woodlands, TX; Baylor Sammons Cancer Center, Dallas, TX; New York Oncology Hematology, Albany, NY; Rocky Mountain Cancer Center, Denver, CO; Physician, New York, NY; Virginia Cancer Specialists, PC, Fairfax, VA; Birmingham Hematology and Oncology, Birmingham, AL; Arizona Oncology Associates, Sedona, AZ; Texas Oncology, Houston, TX; Comprehensive Cancer Center; Henderson, NV; Virginia Oncology Associates, Virginia Beach, VA; Texas Oncology, Plano, TX; Texas Oncology, El Paso, TX

**Introduction:** TC is an effective adjuvant chemotherapy regimen in early stage breast cancer (ESBC) patients. Jones et al (J Clin Oncol 2009 Mar 10;27(3):1177-1183) demonstrated a significantly superior 5-year DFS and OS for TC compared to AC. Because TC was superior to AC in a subset analysis of HER2+ breast cancer treated without trastuzumab, we tested TC in combination with 1 year of trastuzumab in lower risk HER2+ ESBC, predominately node negative disease.

**Methods:** This was an open-label, phase II study of TC+H in HER2+ patients based on HER2 overexpression determined at the local level. Tissue was collected for central review of HER2 and TOP2A status. There was no lower limit of tumor size for lymph node negative cancers. Every 21 days, patients received docetaxel (T) 75mg/m² IV, plus cyclophosphamide (C) 600mg/m² IV, plus weekly trastuzumab (H) 4mg/kg IV (loading dose) and 2mg/kg IV thereafter for a total of 4 cycles. After 4 cycles of TC+H, patients continued on H for a total of 1 year. Appropriate patients received radiotherapy. Cardiotoxicity (decreased left ventricular ejection fraction) was assessed by MUGA or ECHO at baseline, at completion of TC+H, and then at 3-month intervals until completion of trastuzumab treatment. The primary endpoint was DFS at 2 years with continued follow-up for 3 years. Secondary endpoints were OS and safety.

**Results:** 486 patients received treatment, and 397(82%) completed a full year of H. Median age was 55 years (range: 24-75.8), ECOG 0-1, and 95% of patients were Stage II, and 6(1.2%) were Stage III. Disease-free survival and OS at 2 and 3 years are listed in the table below according to various features.

<table>
<thead>
<tr>
<th>Table 1</th>
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<tr>
<td>No. Patients</td>
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<tr>
<td>444 safety population</td>
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<tr>
<td>101 node positive</td>
</tr>
<tr>
<td>385 node negative</td>
</tr>
<tr>
<td>385 node positive</td>
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<tr>
<td>106 &gt;1.1 cm tumor size</td>
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<tr>
<td>221 1.1-2.0 cm tumor size</td>
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<td>139 &gt;2.0 cm tumor size</td>
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The most common Grade 3/4 toxicities were neutropenia (47.1%) and febrile neutropenia (6.2%). There were 5 deaths: 1 due to PD, 1 aspiration, and 1 respiratory distress syndrome (possibly related to treatment), 1 cardiopulmonary arrest, and 1 unknown. There were 13 cases of recurrent breast cancer (local 5, or local/distant 8). Cardiac dysfunction occurred in 28(5.8%) patients, with 12(2.5%) being Grade 1, 14(2.9%) Grade 2, and 2(0.4%) grades 3/4. Sixteen patients had to stop H due to cardiac dysfunction with H. Central assessment of TOP2A status was performed by FISH in 90% of cases: TOP2A-amplified (42%), deleted (27%), and normal (31%). However TOP2A status had no effect on outcome.
Conclusions: 1) The HER TC regimen was evaluated in 486 patients with HER2 overexpressed ESBC and found to be effective at the primary endpoint of 2 years. 2) Efficacy was demonstrated in node negative cancer including 94 cancers <1 cm. 3) Toxicity was acceptable with a low rate of cardiac dysfunction, mainly reversible. 4) The HER TC regimen is an option for patients with lower risk HER2 overexpressing ESBC.

PD07-04
Lapatinib vs Trastuzumab in Combination with Standard EC-D Chemotherapy in the Neoadjuvant Treatment of HER2+ Patients.

Results from the GEICAM 2006-14 Phase II Randomized Trial. Alba E, Albarello J, de la Haba J, Barnadas A, Calvo L, Sánchez P, Ramos M, Rojo F, Burgués O, Porras I, Tibau A, Carrasco E, Cámara MC, Lluch A. Hospital Virgen de la Victoria, Malaga, Spain; Hospital del Mar, Barcelona, Spain; Hospital Universitario Reina Sofia, Córdoba, Spain; Hospital Santa Creu i Sant Pau, Barcelona, Spain; Complejo Hospitalario Universitario de A Coruña, A Coruña, Spain; Hospital General de Jaén, Jaén, Spain; Centro Oncológico de Gálicia, A Coruña, Spain; Fundación Jiménez Díaz, Madrid, Spain; Hospital Clínico Universitario de Valencia, Valencia, Spain; GEICAM (Spanish Breast Cancer Research Group), San Sebastián de los Reyes, Madrid, Spain.

Background: The addition of trastuzumab (T) to neoadjuvant chemotherapy increases pCR rate in patients with HER2+ breast cancer. Lapatinib (L) in combination with chemotherapy is also an active drug in breast cancer patients. This study investigates the efficacy of trastuzumab or lapatinib added to chemotherapy in the neoadjuvant setting.

Methods: Patients (P) with tumors greater than 2 cm (or less with positive axilla) and HER2+ (Herceptest 3+ or 2+ with FISH+) that have not received any prior treatment for breast cancer were recruited. Pts were randomized to receive epirubicin 90 mg/m2 plus cyclophosphamide 600 mg/m2 x 4 cycles (cy) followed by docetaxel 100 mg/m2 (with growth colony stimulating factor support) plus either T 8 mg/kg loading dose and then 6 mg/Kg intravenous (EC-DT) or L 1250 mg orally (EC-DL). Patients were stratified according to tumor size (T1-2 vs T3-4), and estrogen receptor status (positive vs negative). The primary end-point was pathological complete response (pCR) in the breast measured by Miller and Payne criteria. Secondary end-points were clinical response (by imaging test), toxicity and correlation between pCR and tumor biomarkers.

Results: From February-09 to October-10, 102 pts were randomized (50 EC-DT, 52 EC-DL). Pts characteristics were well balanced between arms. Median age was 48 years (30-79), 56% of pts were premenopausal. 7% grade I, 45% grade III. 14/59/12/15% were T1/T2/T3/T4 and 69% N+. 58% were estrogen receptor positive and 44% progesterone receptor positive. Three patients were FISH negative after central assessment and were not considered evaluable for efficacy, pCR rate in the breast was 52% (95% CI: 38-66) for EC-DT and 25% (95% CI: 13.5-37.5) for EC-DL (p-value=0.0065). pCR---pN0 was 48% (95% CI: 34-62) for EC-DT and 24% (95% CI: 12 – 35) for EC-DL (p-value=0.01). Clinical response was 77% with EC-DT and 65% with EC-DL (p-value=0.176). Grade III-IV toxicity was similar between arms except for diarrhea that was more frequent in EC-DL 14% than in EC-DT 2% (p-value=0.03); more patients discontinue treatment due to toxicity with EC-DL (1 vs 6; p-value=0.055).

Conclusions: Our study shows that EC-DT was more efficacious and less toxic than EC-DL in the neoadjuvant treatment of HER2+ pts. Biomarker profiling in the tumors, including activation levels of tyrosine-kinase receptors, signaling pathways and surrogate markers (proliferation and apoptosis) is ongoing; their correlation with pCR will be presented at the meeting.

PD07-05
Local Recurrence Risk in 6377 Patients with Early Breast Cancer Receiving Neoadjuvant Anthracycline-Taxane +/- Trastuzumab Containing Chemotherapy.


Background: Locoregional recurrence (LRR; defined according to Hudis C, JCO 2007) risk after neoadjuvant systemic treatment is considered as a possible hazard of this treatment approach. However, few data exist on the incidence and risk factors for LRR after anthracycline-taxane +/- trastuzumab (AT +/- H) containing neoadjuvant treatment. We analyzed individual data of 7 prospective neoadjuvant trials conducted by the German Breast Group and the AGO Breast Group.

Patients (Pts) and methods: 6377 Pts with operable or locally advanced, non-metastatic breast cancer were analyzed (for details see von Minckwitz G et al, BCRT 2010). Postsurgical radiotherapy was indicated after breast conservation for all patients and after mastectomy for patients with cT3/4 or cN+ disease. Endocrine treatment was given to ER- and/or PgR-positive patients. 485 LRR were observed during a median follow up of 46.2 (0-127) months.

Results: LRR was similar for patients treated by tumorectomy (7.2% of N=1123), segmentectomy (6.8% of N=1112), quadrantectomy (7% of 557), or breast conservation (BCT) (not otherwise specified) (7.7% of N=819), but higher in patients treated by mastectomy (ME) (12.1% of N=1670) (p<0.001). Rate of breast conservation decreased by increasing initial tumor size (cT1(N=198): 77.7%, cT2(N=3675): 78.1%, cT3(N=795): 49.4%, cT4a-c(N=348): 35.9%, cT4d(N=235): 19.1%). LRR in patients treated by BCT or ME were 9.1% vs 9.1% for cT1 (p=0.9), 6.9% vs. 9.8% for cT2 (p=0.001); 9.7% vs 14.2% for cT3 (p=0.04); 3.2% vs. 11.7% for cT4a-c (p=0.004; and 22.2% vs 18.9% for cT4d (p=0.4). LRR increased with surgical yT-stage from 4.7% for ypT0 (N=990), 11.8% for ypTis (N=340), 9.1% for ypT1 (N=1555), 8.2% for ypT2 (N=926), 13.8% for ypT3 (N=232), 20% for ypT4a-c (N=80), to 31.2% for ypT4d (N=16) (p<0.001). Comparable results were obtained for cN and cNp stages. Patients with a pathological complete response (pCR = ypT0 ypN0) showed a lower LRR of 3.7% compared to patients not achieving a pCR (3.7% vs 9.9% (HR 0.36 p<0.001). Patients with a pCR showed low LRR in all intrinsic subtypes except Luminal B/HER2- like tumors (Luminal A-like tumors (N=105; 3.8%), Luminal B/HER2- like (N=40; 0%), Luminal B/Her2+ (N=124; 8.1%), HER2+ (non-luminal)-like (N=158; 1.9%), triple-negative (N=276; 2.5%) (p=0.016). Patients without a pCR showed an excessive LRR for HER2+ (non-luminal) and triple-negative tumors (Luminal A-like
tumors (N=1498; 5.1%), Luminal B/HER2-like (N=304; 11.9%), Luminal B/HER2+ like (N=602; 8.5%), HER2+(non-luminal)-like (N=367; 18%) and triple-negative (N=276; 17.8%) (p<0.001). cT, cN, ypN, intrinsic subtype, but not ypT stage and type of surgery were independent predictors of LRR for patients without pCR in a Cox regression model. None of these factors except Luminal B/HER2+ (p=0.012) were significant in patients with pCR.

**Conclusions:** LRR in this large pooled analysis occurring after AT+/-H containing neoadjuvant treatment appears to be low, especially in all patients with a pCR except Luminal B/HER2+. In patients without a pCR low cT, cN, ypN and Luminal tumor type predict a low LRR. Other stages and subtypes without pCR should be carefully followed up irrespective of type of surgery.

**PD07-06**

**Adjuvant Chemotherapy with or without Darbepoetin alpha in Node-Negative Breast Cancer: Survival and Quality of Life Analysis from the Prospective Randomized WSG ARA Plus Trial.**

Nitz U, Glue O, Oberhoff C, Reimer T, Schumacher C, Hackmann J, Warm M, Uele C, Runde V, Kuemmel S, Zana J, Harbeck N, West German Study Group, Moenchengladbach, Germany; Bethesda Hospital, Moenchengladbach, Germany; Bethesda Hospital, Wuppertal, Germany; University Hospital Essen, Essen, Germany; Catholic Hospital Essen North, Essen, Germany; University Hospital Suedstadt, Rostock, Germany; St. Elisabeth Hospital, Cologne, Germany; Marien-Hospital Witten, Witten, Germany; University Hospital Cologne, Cologne, Germany; Krankenhaus Koeln-Holweide, Cologne, Germany; Gynecoological Practice, Hildesheim, Germany; Wilhelm-Anton-Hospital Goch, Goch, Germany; Hospital Essen-Mitte, Essen, Germany

**Background:** Darbepoetin alpha (ARA) is currently used to reduce chemotherapy-associated anemia (CAA) rates in various solid tumors. A possible negative impact of ARA on patient survival has been suggested in some clinical trials. The objective of the prospective randomized phase III ARA Plus trial is to compare the survival effect of darbepoetin alpha use (ARA+/ARA-) in combination with modern standard adjuvant chemotherapy targeting guideline-recommended HH-levels in high-risk breast cancer (BC).

**Methods:** ARA Plus compared 6 cycles T90 A10 C825 q3w or 6 cycles F180 E100 C50 q3w (at discretion of each center) in patients with node positive BC (aged 18-65 years). Patients were randomized to darbepoetin (ARA+) 500 μg q3w until completion of radiotherapy or to standard supportive care (ARA-). ARA was started at Hb-levels ≥13 g/dL (amendment 01/2008: Hb <12 g/dL) and stopped at >14 g/dL. Primary endpoint is event-free survival (EFS: relapses, death without disease evidence, second malignancy). Overall survival (OS), toxicity, Hb-levels and quality of life are secondary endpoints. Survival analysis was planned after 7 years of study duration. EFS was compared with a log-rank test (α=0.05) with a statistical power of 80% and log-rank test. Quality of life was measured using FACT questionnaires at beginning of therapy, mid, end of therapy, and at 1 year afterwards.

**Results:** 1234 pts (616 ARA+/618 ARA-) from 70 centers in Germany were randomized between 01/04 and 06/08. 1198 intent to treat patients (ITT) were analysed (1096 TAC; 102 CEF). Baseline characteristics were well balanced in ARA+ and ARA- arms: median age 53/53 years; tumor size 2.4/2.4cm; number of + LN 3/3; HR+ 80%/ 83.5%, G3 40%/36.7%. Toxicity data have been reported earlier (SABCS 2008).

At median follow up of 40 months, 168 events (81 ARA+, 83 ARA-) and 134 relapses (65 ARA+, 69 ARA-) were reported. There was no significant difference in 3-year EFS between ARA+ and ARA- arms (89.2% vs. 87.6%, p=0.97, χ2-test). 37 deaths were reported in the ARA- and 36 in the ARA+ arm. 3-year OS was 95.4% and 95.1% for ARA+ and ARA-, respectively (p=0.85). Only nodal involvement (≥4 vs. 1-3), negative HR, tumor size ≥2 cm and G3 were significant survival predictors by multivariate analysis. Unplanned retrospective analysis revealed better EFS for ARA+ vs. ARA- in HR- (p=0.05), and no difference in HR+ group (p=0.6). In ARA+ patients, Hb-levels were stable over the whole treatment period with rare overstimulation. In ARA- patients, Hb-levels decreased during therapy (median of all cycles ARA+/ARA-: 12.5/11.6 g/dL). There was no correlation between mean Hb-levels and survival in either study arm. There were no significant differences in mean FACT scores changes (general, anemia, cognitive) from begin to end of therapy in either study arm. More detailed analyses are ongoing.

**Conclusions:** To date, the WSG ARA plus trial is the only prospectively randomized trial in early high-risk BC exclusively focusing on the impact of adjuvant ARA on patient outcome. Supportive administration of ARA appears to be safe and to have no significant survival effect when used in combination with TAC or CEF according to current guidelines.

**PD07-07**

**Combination of Paclitaxel and Bevacizumab without or with Capcitabine as First-Line Treatment of HER2-Negative Locally Recurrent or Metastatic Breast Cancer (LR/MBC): First Results from a Randomized, Multicenter, Open-Label, Phase II Study of the Dutch Breast Cancer Trialists’ Group (BOOG).**

Lam SW, de Groot SM, Honkoop AH, Jager A, ten Tije AJ, Bos MM, Emmink SC, van den Bosch J, Nortier J, Brau J, de Graaf H, Portielje J, Los M, Gooyster DD, van Tinteren H, Boven E, VU University Medical Center, Amsterdam, Netherlands; Comprehensive Cancer Centre the Netherlands, Netherlands; Isala Clinics, Zwolle, Netherlands; Erasmus Medical Center-Daniel den Hoed Cancer Center, Rotterdam, Netherlands; Tergooi Hospitals, Hilversum, Netherlands; Reinder de Graaf Hospital, Delft, Netherlands; The Netherlands Cancer Institute-Antoni van Leeuwenhoek Hospital, Amsterdam, Netherlands; Albert Schweitzer Hospital, Dordrecht, Netherlands; Leiden University Medical Center, Leiden, Netherlands; Vlietland Hospital, Schiedam, Netherlands; Medical Center Leeuwarden, Leeuwarden, Netherlands; Haga Hospital, The Hague, Netherlands; St. Antonius Hospital, Nieuwegein, Netherlands; Franciscus Hospital, Roosendaal, Netherlands

**Background:** First-line treatment of HER2-negative LR/MBC with paclitaxel (T) and bevacizumab (A) has demonstrated improved progression-free survival (PFS) and overall response rate (ORR) when compared with T alone (E2100). We determined whether addition of capcitabine (X) to AT is safe and would be better effective than AT in women with HER2-negative LR/MBC.

**Methods:** Eligibility criteria were age ≥18 & ≤75 years, measurable or non-measurable HER2-negative LR/MBC, ECOG PS 0–1 and no prior chemotherapy for LR/MBC. Patients were randomized in 1:1 ratio to receive AT (4-week cycle of T 90 mg/m2 on days 1, 8, 15 and A 10 mg/kg on days 1, 15 for 6 cycles, followed by A 15 mg/kg on day 1 given 3-weekly for subsequent cycles) or ATX (3-week cycle of T 90 mg/m2 on days 1, 8, A 15 mg/kg on day 1 and X 825 mg/m2 bid on days 1–14 for 8 cycles, followed by A 15 mg/kg on day 1 and X 825 mg/m2 bid on days 1–14 given 3-weekly for subsequent cycles). Treatment was discontinued at disease progression, unmanageable toxicity or withdrawal of consent. The primary endpoint was PFS.
Secondary endpoints were overall survival, ORR, duration of response and toxicity. Efficacy was evaluated according to RECIST 1.0 and toxicity was assessed according to NCI CTCAE 3.0.

**Results:** From June 2007 till December 2010, 312 patients were recruited at 36 sites. The median age was 56 years (range 32–76). Among all patients, 52% had ECOG 0, 85% were hormone-receptor positive, 86% had measurable disease and 8% had bone-only metastases. These factors were well balanced between both arms. A total of 48% and 33% of patients, respectively, received prior hormonal therapy or radiotherapy for LR/MBC. At the data cut-off of 1st June 2011, the median follow-up duration was 23 months. 311 patients received at least one cycle of treatment and were evaluable for safety. The median number of treatment cycles in AT was 9 and in ATX was 11 (both 33 weeks). An ORR of ≥40% was reached in patients with measurable disease in both groups. The incidence of serious adverse events (SAEs) was 47% and 40% for AT and ATX, respectively, while that of treatment-related SAEs was 12% and 19%, respectively. Treatment-related deaths were 2% for AT and 2% for ATX. The overall rate of AEs grade 3 or 4 was similar in both arms as shown in Table 1, except for hand-foot syndrome grade 3 and neutropenia grade 3 in ATX. In addition, 6 patients with pulmonary embolism were reported in ATX.

**Conclusions:**
ATX was well tolerable, although more patients experienced hand-foot syndrome grade 3 and thromboembolic events than patients treated with AT. The efficacy data will be presented at the meeting.
Support: This study was supported by Roche.

**PD07-08**

The Effect on Surgical Complications of Bevacizumab Added to Neoadjuvant Chemotherapy; NSABP Protocol B-40.

Bauer HD, Tang G, Rastogi P, Geyer CE, André R, Atkins JN, Baez-Diaz L, Brufsky AM, Mehta RS, Fehrenbacher L, Pajon ER, Senecal FM, Gaur R, Margoese RG, Adams PT, Gross HM, Costantino JP, Swain SM, Mamounas EP, Wolmark N. National Surgical Adjuvant Breast and Bowel Project (NSABP); Virginia Commonwealth University, Massey Cancer Center; University of Pittsburgh Graduate School of Public Health; University of Pittsburgh Cancer Institute School of Medicine; Allegheny General Hospital; Centre Hospitalier de l’Université de Montréal; Southeast Cancer Control Consortium CCOP; San Juan MBCCCOP; University of Pittsburgh Magee Women’s Hospital; University of California at Irvine, School of Medicine, Chao Family Comprehensive Cancer Center; Kaiser Permanente, Northern California; CCOP, Colorado Cancer Research Program; CCOP, North-West Medical Specialties; Kansas City Clinical Oncology Program; Jewish General Hospital, McGill University; Genesys Regional Medical Center; Dayton CCOP; Washington Cancer Institute, Washington Hospital Center; Aultman Health Foundation

The NSABP trial B-40 was designed to determine whether adding capcitabine or gemcitabine to docetaxel followed by AC would increase the pathologic complete response (pCR) rates in patients with operable HER2-negative breast cancer and to determine whether the addition of bevacizumab to three docetaxel-based regimens followed by AC would increase pCR rates. Secondary endpoints include the percentage of surgical complications after mastectomy, lumpectomy, and axillary staging procedures in the patients who received chemotherapy versus those who received chemotherapy and bevacizumab.

**Methods:** Women with HER2-negative operable breast cancer received one of the following docetaxel-based regimens with or without bevacizumab (15mg/kg) every 3 weeks for four cycles: docetaxel 100 mg/m² IV; docetaxel 75 mg/m² and capcitabine 825 mg/m² po BID days 1-14; and docetaxel 75 mg/m² day 1 and gemcitabine 1000 mg/m² IV days 1 and 8. These regimens were followed by preoperative standard AC on day 1 every 21 days for four cycles with or without bevacizumab 15 mg/kg given with the initial 2 cycles of AC. Treatment groups randomized to receive bevacizumab resumed postoperative bevacizumab 15 mg/kg IV every 3 weeks for an additional 10 doses. Assessment of surgical complications was performed from the date of surgery through 24 months following study entry.

**Results:** Planned analysis for surgical complications will be conducted in October 2011 and will be presented along with breast reconstruction data.

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**PD08-01**

JNK2 Regulates Mammary Lineage Differentiation in Tumors and Normal Glands through Notch1 and p53.

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The classification of patient tumors by clinical subtype has gained wide acceptance due to the implications for prognosis and treatment. However, recent studies have cast doubt on previously advocated normal mammary origins of these subtypes. Thus, the link between the normal mammary gland and mammary tumors is more complex than expected. C-Jun N-Terminal Kinase-2 (JNK2) is a protein that is involved in numerous developmental processes and our previous work has shown it to be important for DNA damage response in mammary tumors. In attempt to gain insight into the link between mammary development and tumorigenesis, we compared normal mammary glands of JNK2 knockout (jnk2ko) mice to jnk2ko mammary tumors expressing or lacking wildtype p53 (p53ko). These studies showed that jnk2ko glands possess 35% fewer basal cells (p=0.0078) with a corresponding increase in luminal epithelial cell populations (p=0.100). This luminal response is corroborated by in vitro 3D assays of primary mammary epithelial cells (MECs) where luminal cell differentiation is normalized by inhibition of Notch signaling. Expression notch-1, a well-known regulator of MEC differentiation, is increased jnk2ko mammary glands. Increased expression of the Notch-1 target gene, hes-1, was also seen (p=0.005). Histology revealed that increased expression of active Notch-1 is localized to the mammary stem cell niche, the terminal end bud. Similar to the normal gland, jnk2ko mammary tumors possessing wildtype p53 exhibit decreased proportions of basal cells (p=0.0002) and increased proportions of luminal cells (p=0.0411) relative to wildtype. Jnk2ko cell lines derived from these tumors show decreased expression of notch-1 (p=0.0018) and hes-1 (p=0.0602) following introduction of JNK2. Luciferase assays comparing activity of the notch-1 promoter to a notch-1 promoter with mutated p53 response elements revealed...
a dependence of increased notch-1 promoter activity in jnk2ko cells on the p53 response element. P53ko tumor cells, by contrast, do not exhibit alterations in notch-1 promoter activity in the absence of p53 response elements, regardless of JNK2 status. QPCR showed that loss of JNK2 in normal mammary glands and tumors causes increased p53 expression—thus providing a potential mechanism. In support that Notch upregulation in the absence of JNK2 is dependent upon p53, normal glands lacking p53 show no differences in lineage differentiation. P53ko tumors also show no differences in basal lineage differentiation, however, increases in luminal differentiation are maintained in the absence of JNK2. Consistent with increased luminal differentiation, jnk2ko caused decreased expression of markers involved in the epithelial to mesenchymal transition phenotype. This data suggests that JNK2 is important not only for lineage differentiation in normal mammary glands, but in mammary tumors and that the effect is dependent on both Notch1 and p53.

PD08-02
Targeting BCL-2 Expressing Breast Tumors with BH3-Mimetics – A New Class of Drugs in Breast Cancer?
Lindeman GJ, Oakes SR, Vaillant F, Lim E, Lee L, Breslin K, Feleppa F, Deb S, Ritchie ME, Takano E, Ward T, Fox SB, Generali D, Smyth GK, Strasser A, Huang DCS, Visvader JE. The Walter and Eliza Hall Institute of Medical Research, Parkville, VIC, Australia; The Royal Melbourne Hospital, Parkville, VIC, Australia; The University of Melbourne, Parkville, VIC, Australia; Peter MacCallum Cancer Centre, East Melbourne, VIC, Australia; Ospitalieri di Cremona, Cremona, Italy

**Background:** Impairment of apoptosis is a hallmark of cancer and can result in resistance to chemotherapy. Tumor resistance to apoptosis is frequently acquired through deregulated expression of BCL-2 family members or inactivation of the p53 tumor suppressor pathway. Over-expression of the pro-survival protein BCL-2 is common in breast cancer (where it is readily detected by immunostaining), and has been shown to be an important prognostic marker. A potential role for BCL-2 as a *therapeutic target* in breast cancer, however, has not been explored. Recently, small molecules termed ‘BH3-mimetics’ have been developed that mimic the action of pro-apoptotic BH3-only proteins. These bind and neutralize pro-survival proteins including BCL-2. Here we have derived a panel of primary breast tumor xenografts (that include basal-like breast tumors) to study the efficacy of the BH3-mimetic ABT-737 combined with docetaxel in targeting BCL-2-positive breast cancer.

**Methods and Results:** We first studied the expression of BCL-2, pro-survival family members BCL-XL and MCL-1, and the pro-apoptotic protein BIM in tissue microarrays of 197 primary breast tumors, which were subclassified on the basis of ER, PR, HER2, CK5/6 and EGFR expression. BCL-2 was overexpressed in luminal (83.3%), HER2-positive (50.0%), basal-like (18.5%) and ‘marker-null’ (41.4%) breast cancers. BCL-2-positive tumors generally co-expressed BCL-XL (96.2%), MCL-1 (94.7%) and BIM (93.5%).

To determine whether the BH3-mimetic ABT-737 (which neutralizes BCL-2, BCL-XL and BCL-W) was effective in targeting BCL-2-expressing breast tumors, we generated a panel of 28 primary breast tumor xenografts in immunocompromised NOG mice. Five xenograft lines (838T, 24T, 315T, 13T and 806T) were selected for further analysis. Four were basal-like, and one (315T) was a luminal B tumor, as determined by gene profiling. Mice bearing tumor xenografts were treated with ABT-737 (50 mg/kg i.p. d1-10), docetaxel (10 mg/kg i.p. d1) or a combination in q21d cycles. Tumor response and overall survival were significantly improved by combination therapy, but only for tumors that expressed elevated levels of BCL-2. Treatment with ABT-737 alone was ineffective, suggesting that ABT-737 sensitized tumors to docetaxel. Combination therapy was accompanied by a marked increase in apoptosis and dissociation of BIM from BCL-2, indicating that a perturbation of BIM complexes may contribute to the activation of the apoptotic cascade. Notably, ABT-737 appeared effective in targeting BCL-2-expressing basal-like tumor xenografts (838T and 24T) harboring p53 mutations.

**Discussion:** Primary breast tumor xenograft models that recapitulate the phenotype of the primary tumor have been developed as useful ‘proof-of-principle’, pre-clinical models. Here we provide the first *in vivo* evidence that BH3-mimetics can be used to sensitize primary BCL-2-expressing breast tumors to taxane chemotherapy. Our results suggest that elevated BCL-2 expression constitutes a predictive response marker in breast cancer. These findings provide a rationale for the development of clinical protocols using the oral analogue ABT-263 (navitoclax) as an adjunct to taxane chemotherapy in BCL-2-expressing basal-like and luminal breast cancer.

**PD08-03**
Inhibition of MEK/ERK- and JNK-Dependent Expression of Interleukin-6 and Interleukin-8 Targets Basal-Like Breast Cancer Stem Cells.
Balko JM, Cook RS, Kuba MG, Miller TW, Bhola NE, Sanders ME, Mezeyo IM, Dowsett M, Gomez H, Arteaga CL. Vanderbilt University, Nashville, TN; Royal Marsden Hospital, United Kingdom; Instituto Nacional de Enfermedades Neoplasicas, Lima, Peru

**Background:** Neoadjuvant chemotherapy (NAC) induces a pathological complete response in approximately 30% of triple-negative or basal-like breast cancers (BLBC). However, patients with residual disease often recur after surgery, presumably due to persistent drug-resistant subpopulations with cancer stem cell (CSC)-like properties. Thus, elimination of this CSC compartment in BLBC has the potential to improve survival by reducing post-surgical metastatic recurrences.

**Methods:** We sampled 49 post-NAC breast cancer samples including 22 BLBCs and quantified RNA for 355 cancer-related transcripts using Nanostring technology. Transcripts associated with a high Ki67 in the residual disease (a biomarker of early recurrence) were identified and bioinformatically examined for an association with drug resistance and a CSC phenotype. Loss of DUSP4, a negative feedback regulator of ERK1/2 and JNK1/2, was highly associated with a high post-NAC Ki67. We examined the role of loss of DUSP4 in promoting a drug-resistant, CSC phenotype.

**Results:** Low DUSP4 expression in post-NAC tumors was associated with high ERK1/2 activation and BLBC gene expression. siRNA knockdown of DUSP4 enhanced resistance to anti-cancer chemotherapy (docetaxel, camptothecin). Alternatively, forced DUSP4 expression in breast cancer cell lines abrogated the activation of transcription factors downstream of ERK and JNK, and sensitized cells to docetaxel-induced apoptosis. In highly metastatic BLBC cell lines, MEK inhibition with AZD6244 and JNK inhibition with SP600125 significantly reduced mammosphere formation and self-renewal. Inhibition of JNK and MEK reduced DUSP4 expression in breast cancer cell lines, indicating that a perturbation of DUSP4 expression in breast cancer cell lines may be a novel mechanism to target breast cancer stem cells.
expression in a cohort of 113 post-NAC triple negative breast cancers. Conclusions: Our data demonstrate that loss of DUSP4 in BLBC promotes ERK and JNK activation by impairing negative feedback of these pathways. Activation of ERK and JNK drives expression of IL6 and IL8 expression, possibly through ETS-1 activation. Thus, targeting these signaling pathways may eliminate residual cancer stem cells after neoadjuvant chemotherapy and improve cure rates in BLBC.

**PD08-04**

**Inhibition of the TGFβ/TGFβR2 Pathway Prevents Enrichment of Drug-Resistant Breast Cancer Stem Cells by Anti-Cancer Chemotherapy.**

**Bhola N, Arteaga C. Vanderbilt University Medical Center; Nashville, TN**

Triple-negative breast cancer (TNBC) is the most virulent form of breast cancer and is associated with a worse prognosis compared to hormone receptor- and HER2-positive tumors. The standard treatment of TNBC is cytotoxic chemotherapy. TNBC patients tend to display a good initial response to chemotherapy; however, they exhibit higher recurrence rates and overall poor long-term survival. Reasons for this high metastatic recurrence rate and mortality are believed to be the existence of a chemo-resistant tumor-initiating population called cancer stem cells (CSCs). CSCs are defined as cells with the ability to self-renew, differentiate into non-tumorigenic cells, and initiate tumors in vivo. CSCs isolated from breast cancer tissue display increased transforming growth factor (TGF) β and TGFβ type II receptor (TGFβR2) mRNA expression compared to non-NCSC population. Furthermore, breast CSCs exhibit characteristics of an epithelial to mesenchymal transition (EMT), a process driven by the TGFβ signaling and associated with metastatic progression. Therefore, we hypothesize that inhibiting the TGFβ/TGFβR2 pathway can abrogate the breast CSC population and sensitize TNBC to chemotherapy, thus reducing metastatic recurrences and tumor progression.

To address this hypothesis, we used the TNBC cell lines SUM159 and BT549. We examined the effect of TGFβ1, the TGFβ receptor I/II kinase inhibitor LY2157299, the TGFβRII receptor and TGFβ1 neutralizing antibodies TR1, and LY2424087 and paclitaxel on the CSC population of these cell lines by 1) flow cytometric analysis of stem cell markers (ALDH, CD44, and PROCR); and 2) mammosphere formation assays. In both cell lines, LY2157299, LY2424087, and TR1 abrogated TGFβ-mediated CSC enrichment and mammosphere formation (p≤0.01). Treatment with paclitaxel resulted in an enrichment of CSCs as measured by FACS and mammosphere formation (p≤0.005) both in vitro and in SUM159 xenografts established in athymic mice. Further, treatment with paclitaxel upregulated TGFβR2 and TGFβ1 mRNAs and phosphorylated SMAD2 levels in CSCs sorted from post-therapy SUM159 xenografts. RNAi-mediated knockdown of TGFβR2 in BT549 cells decreased mammosphere formation but did not affect the proportion of CSCs analyzed by FACS (p≤0.005 and p=0.03, respectively). TGFβR2 and SMAD4 siRNA decreased the CD44+/CD24+ population in both cell lines. SMAD4 siRNA also increased CSCs, mammosphere formation, and viability in SUM159, BT549, and MDA-231 basal-like and HCC1954 (HER2+) cells. SMAD4 knockdown resulted in a significant decrease in the CSC-associated genes interleukin-8 (IL-8) and Nanog. Finally, a combination of either LY2157299 or SMAD4 siRNA with paclitaxel decreased both stem cell marker expression, IL-8 and mammosphere formation compared to each treatment alone (p≤0.006).

These findings suggest that autocrine TGFβ signaling plays a maintenance role in breast CSCs viability. Second, blockade of the TGFβ/TGFβR2 pathway with genetic or pharmacological inhibitors can ameliorate or prevent the enrichment of drug-resistant CSCs by anti-cancer chemotherapy. These studies provide a rationale for studies of chemotherapy ± TGFβ inhibitors in patients with TNBC using stem cell markers as surrogates of clinical response.

**PD08-05**

**Stat3 Signaling in Human Breast Cancer Stem Cells.**

**Wei W, Zhang M, Tweardy D, Rosen J, Lewis M. Baylor College of Medicine**

Recent data suggest the existence of a unique subset of breast cancer cells capable of initiating tumor growth and giving rise to all other cells characteristic of a given tumor. These cells have been termed “cancer stem cells” or “tumor-initiating cells”. Breast cancer stem cells appear to be resistant to chemo- and radiotherapies and may be responsible for recurrence and metastasis in breast cancer patients. Identifying the cellular signaling pathways responsible for breast cancer stem cell maintenance and self-renewal represents a critical hurdle for developing effective therapeutics.

The Stat3 pathway is a critical regulator of the function of normal stem cells, and shows altered expression in human breast cancers [4]. Moreover, IL-6, the Stat3 signaling agonist, is required for breast cancer stem cell function in human breast cancer cell lines. All these suggest an important role of Stat3 signaling in breast cancer stem cells. However, due to lack of method for pathway-activity-based live cell separation, whether Stat3 functions in the cancer stem cells themselves or whether it may function in surrounding niche cells remain unknown. We have constructed a lentiviral fluorescent reporter for Stat3 signaling which contains four copies of M67 Stat3 binding sites upstream of enhanced Green Fluorescent Protein. This reporter system enables FACS-sorting of cells with active Stat3 signaling and in vivo/in situ localization of Stat3 responsive cells. In addition, C188-9, the first Stat3 specific competitive inhibitor, has been developed in our collaborator’s lab, which provides us an advanced tool in studying Stat3 function.

We hypothesize that Stat3 signaling is preferentially active in stem-like subpopulation and depend on non-stem cancer cells to maintain its activation. Three aims to test this hypothesis include: 1. To test whether cancer cells with activated Stat3 signaling are enriched for breast cancer stem cells or whether they may serve as niche cells. 2. To test whether small molecule antagonists of Stat3 signaling can inhibit cancer stem cell function. 3. To identify novel targets of Stat3 signaling that may serve as indicators of responsiveness to Stat3 antagonist or predict treatment response.

So far, our GFP reporters for Stat3 signaling effectively report Stat3 activity in both patient xenografts and human breast cancer cell lines in vivo and in vitro, which enables effective separation of Stat3+ cells for functional study. MDA231 and SUM159 tumors with reporters have been established. Tumor cells will be sorted according to Stat3 activity and perform limiting dilution and MSFE assays. Tumors with reporters will also be treated by C188-9, Chemo, or in combination to study synergistic effect of Stat3 inhibitors on tumor growth. Preliminary data based on inhibitor and limiting dilution studies suggested that Stat3 signaling is required for stem cell function. However, neither Stat3+ nor Stat3- cells are self-sufficient in performing these functions, indicating communications between subpopulations.
PD08-06
ERK2 Promotes Stem Cell-Like Characteristics in Triple-Negative Breast Cancer.
Bartholomeusz C, Saso H, Daddin A, Kazuharu K, Hortalobygi GN. The University of Texas MD Anderson Cancer Center, Houston, TX
Background: Triple-negative breast cancer (TNBC) is resistant to targeted therapies such as hormonal therapy and HER2-targeted therapies. Thus, no specific targeted therapy is currently available for TNBC. ERK2, a component of the MAPK pathway, plays an important role in epithelial-mesenchymal transition (EMT) in MCF-7-10A, a nontransformed human mammary epithelial cell line and is required for full acquisition of stem cell-like characteristics. Inhibition of ERK2 dramatically reduces cell growth, whereas inhibition of ERK1 significantly facilitates proliferation. ERK1 mutant mice have a strikingly milder phenotype than ERK2 mutant mice, which die early in development. In addition, we have shown that the upstream target of ERK, MEK, may be a target for treating TNBC with the MEK inhibitor selumetinib. Our reverse-phase protein array analysis showed that of patients with TNBC, those with ERK2-overexpressing tumors were at higher risk of death than those with low-ERK2-expressing tumors. Therefore, we hypothesized that ERK2 promotes the tumorigenesis and metastasis of TNBC. We sought to determine the role of ERK2 in the tumorigenic and metastatic activities in TNBC and whether ERK2 is required for acquisition of stem cell-like characteristics in TNBC.
Methods: We studied the role of ERK1 and ERK2 in the TNBC cell line SUM-149. We used shRNA to specifically knockdown ERK1 and ERK2 (shERK1 and shERK2) and examined whether knocking down ERK1 and ERK2 correlated with EMT regulation, migration, and tumorigenicity in TNBC cells.
Results: Compared with parental SUM-149 cells, stable clones that constitutively expressed shERK1 or shERK2 showed no difference in growth rate. However, knocking down ERK2 significantly inhibited migration and the acquisition of stem cell-like characteristics (CD44+/CD24-) in SUM-149 cells in vitro. In addition, knockdown of ERK2 also inhibited anchorage-independent growth, an indicator of in vivo tumorigenicity, whereas knockdown of ERK1 did not inhibit anchorage-independent growth. However, inhibition of ERK1 or ERK2 in 2D or 3D cell culture did not correlate with changes in epithelial and mesenchymal markers by western blot analysis.
Conclusion: Our data demonstrate that ERK2 may promote the tumorigenesis of TNBC via enriching cancer stem cells. Our long-term goal is to develop ERK-targeted therapy for TNBC.

PD08-07
Wound-Healing Drainage Fluids Promote Triple Negative Breast Cancer Progression.
Campiglio M, Sassò M, Bianchi F, Plantamura I, Iorio M, De Cecco L, Giustarini E, Agresti R, Ghibellini C, Crocchetta M, Tripodo C, Tagliafure E. Fondazione IRCCS-Istituto Nazionale Tumori, Milan, Italy; University of Palermo, Palermo, Italy
Triple negative breast cancers (TNBC) account for 15% of breast cancers. TNBCs carry a high risk of recurrence and deaths, due to the high rate of local and systemic relapse in these patients and no therapeutic options except chemotherapy are currently available. The TNBC pattern of recurrence present a distant recurrence peak at approximately 3 years and then declines rapidly thereafter, whereas in all non-TNBC types the recurrence risk seems to be constant over time. TNBC relapse risk is comparable to that of HER2-positive tumors subtype, in which growth-factors released during the healing process accelerate the early recurrences in HER2-positive patients.

Thus, we speculate that also TNBC early relapse may depend on their capability to respond to wound-healing stimulation. To this aim, TNBC were treated with drainages to identify which receptors/pathways can be activated and play a driving role in TNBC progression. A pilot reverse phase protein microarray (RPMA) experiment on MDA-MB-231 TN cells drainage-fluids stimulated revealed a specific activation of PDGFR and VEGFR and their downstream pathways, whereas no significant changes were observed in other receptors, such as EGFR, IRS, Met and ERB3. The type of activated receptors suggested the involvement of endothelial receptors upon drainages stimulation and, indeed TNBC cell lines expressed endothelial molecules, such as CD34, CD31, CD146. Beside the proved role of some of these receptors in cellular proliferation, the TNBC endothelial-like phenotype prompt us to analyzed TNBC cell lines capability to form vascular-like channels when seeded on matrigel. Drainages were able to accelerate the formation of vascular channels in TNBC cell lines and, moreover to consistently increase proliferation of TNBC cells compared to non-TNBC cells. To prove whether receptors found activated by drainages play a key role in TNBC progression, we targeted PDGFR, VEGFR and other receptors possibly involved in angiogenesis and vasculogenic mimicry with sunitinib (targeting PDGFR, VEGFR, FGF and c-kit), anti-bFGF antibody (Ab)(targeting the ligand bFGF) and bevacizumab (targeting the VEGF) in TNBC cells drainage-stimulated in vitro. Sunitinib and anti-bFGF Ab halved the proliferation of TNBC cell lines and reduced of almost 60% the formation of vascular-like channels in TNBC cells, whereas bevacizumab modestly affect proliferation but not vasculogenic properties. Notably, sunitinib and anti-bFGF Ab strongly inhibited MDA-MB-231 and MDA-MB-468 xenografts tumor growth (sunitinib: 80%, and 70% Growth Index (GI), respectively; anti-bFGF Ab 70% and 60% GI, respectively) whereas bevacizumab determined no more than 30% decrease of tumor volume. Unfortunately, all these drugs did not efficiently control the development of lung metastases, that indeed significant increased compared to their control, possibly through induction of hypoxia processes. In conclusion, wound healing promotes TNBC progression by sustaining proliferation and vasculogenesis. The use of sunitinib and anti-bFGF antibody strongly inhibited tumor growth in mice models, but significantly increased lung metastases suggesting a combined use of these drugs with molecules able to interfere with hypoxia pathway.

PD08-08
Preclinical Efficacy of the Combination of Met and Src Family Kinase Inhibitors in Triple-Negative Breast Cancer.
Gartner EM, Kim EMH, Choi L, Boerner J. Karmanos Cancer Center at Wayne State University, Detroit, MI
Background: Epidermal growth factor receptor (EGFR), although overexpressed in almost 60% of all triple-negative breast cancers, has yet to be identified as an effective therapeutic target for the treatment of the disease. We have previously shown in cell culture models of triple-negative breast cancer that primary resistance to EGFR tyrosine kinase inhibitors through ligand-independent phosphorylation can be overcome by co-inhibiting EGFR with either Met or Src family kinases (SKFs). We also demonstrated that inhibiting Met kinase activity reduced EGFR and c-Src phosphorylation and inhibiting SKF reduced EGFR phosphorylation. Taken together, these data suggest that EGFR, Met and SKFs regulate the activity of each other through transphosphorylation. This process of transphosphorylation, or crosstalk, allows for the continued activation of key signaling proteins required for proliferation and transformation. Here we demonstrate that inhibiting both Met and SKFs decreases cell viability.
and eliminates the need for inhibition of EGFR tyrosine kinase activity, as the autophosphorylation sites of EGFR were lost with the combination treatment.

Methods: Seven triple-negative (SUM102, SUM149, SUM229, SUM1315, BT20, BT549, MDA-MB-231), two HER2+ (SUM190 and SKBr3), and two ER/PR+ (MCF7 and T47D) breast cancer cell lines were used to test the efficacy of the combination of Met and SFK inhibitors, Arq-197 and dasatinib, respectively. Cell viability after drug treatment was assessed by MTT assays. IC50 values were calculated and drug synergy calculations were performed. Transphosphorylation was measured by immunoblotting using phospho-specific antibodies.

Aptosis was characterized by immunoblotting.

Results: All seven triple-negative breast cancer cell lines demonstrated decreased cell viability with the combination of Arq-197 and dasatinib when compared with either drug alone. In contrast, none of the HER2+ or ER/PR+ breast cancer cell lines had any additional loss of viability with the combination treatment. We found that the interactions between Arq-197 and dasatinib were synergistic in several of the breast cancer cell lines. Specifically, in one such cell line (BT20), the combinatorial index values were less than 1.0. Interestingly, in this cell line, the tyrosine phosphorylation levels on residues 1068 and 992 of EGFR were significantly altered with the combination treatment and activation of apoptotic proteins, including PARP, occurred.

Conclusions: These data demonstrate that two tyrosine kinases, known to be downstream of EGFR activation, interact to promote resistance to EGFR inhibitors. However, inhibiting both Met and SFKs was sufficient to decrease cell viability and stimulate apoptosis in triple-negative breast cancer cells. Further studies to validate the relevance of these findings are ongoing.

PD08-09
PTPN12 Gene Expression Signature in Triple Negative Breast Cancer Cohort.
Ghazalpour A, Bender RP, McGinniss MJ, Ashfaq R. Caris Life Sciences, Phoenix, AZ

PTPN12 tyrosine phosphatase may play a role in tumor development/progression in triple negative breast cancer patients (TNP). The effects of PTPN12 appear to be mediated through several tyrosine kinase receptors including EGFR, HER2, and PDGFR-beta. We investigated the variability associated with PTPN12 transcript in the microarray gene expression data obtained from 105 TNP as determined by IHC for ER and PR and IHC and FISH for HER2 during our clinical molecular profiling on solid tumors. The mRNA levels of PTPN12 in our cohort was highly variable suggesting a complex genetic regulation of PTPN12 transcription in TNP patients. The highly variable nature of PTPN12 mRNA levels lead us to perform a correlation-based analysis of the transcriptome in TNP samples to gain insight into pathways and cellular processes associated with PTPN12 variation. Specifically, we quantile-normalized and performed two-dimensional hierarchical clustering of the 1000 top correlated genes with PTPN12 expression across the 105 TNP samples. We identified seven distinct gene clusters and three distinct patient subpopulations. The three distinct subtypes of TNP were comprised of low expressing PTPN12 (median log2 expression of 12.3), medium expressing PTPN12 (median log2 expression of 13.1), and high expressing PTPN12 (median log2 expression of 13.4). From the 7 gene clusters identified, 6 were positively correlated and one cluster (cluster 3) was negatively correlated with PTPN12 expression. Upon examining the genes within each cluster, we found that all contain unique set of genes related to cell proliferation, cell death, cell motility, cell cycle regulation and other cancer related pathways. From the 7 clusters, clusters 1, 6 and 7 had the highest fraction of such genes. The gene expression pattern and the gene content of these three clusters is as follows: Cluster 1 (208 genes) were genes that were highly expressed in all three TNP groups and the expression of the genes was highest in high PTPN12 expressing patients. Functional classification of genes by the DAVID bioinformatics tool at NCBI showed several genes related to cancer, including 19 MAP kinase signaling genes, and 15 genes involved in regulation of apoptosis. Cluster 6 (150 genes) contained genes showing very low expression in low-expressing TNP, moderately expressed in medium expressing TNP, and highly expressed in high-expressing TNP. Cluster 7 had the highest fraction of cancer related genes including 7 mitosis and cell cycle check point genes, 6 cytoskeletal genes, 4 phosphatases and 6 kinases including KRAS, 4 DNA repair genes, 8 signal transduction genes, 3 positive regulator of apoptosis, and 22 genes involved in regulation of gene expression. Cluster 7 (166 genes) contained genes that were expressed at low level throughout the three TNP groups but the severity of under-expression was higher in TNP patients with low PTPN12 expression. In cluster 7 there were 5 genes belonging to the EGFR and FGF signaling pathways, and 6 genes involved in mitosis. It is worth noting that EGFR pathway has been postulated to crosstalk with PTPN12 protein. All together our results provide support for the involvement of PTPN12 in cancer development and highlights a promising therapeutic target for TNP patients.

PD08-10
High Frequency of Triple Negative Mammary Carcinomas in the Dog as Model of Human Breast Cancer.
Nguyen F, Abadie J, Loussouarn D, Ibisch C, Rieder N, Campion L, Belousov A, Benelmans I, Hanzenne C, Campone M. Ecole Nationale Vétérinaire-ONIRIS, Site Chantrerie- BP4076, Nantes, France; Roche Diagnostics GmbH; Nonenwald 2, Penzberg, Germany; CHU de Nantes, Site Nord-Laennec, Nantes, France; Institut de Cancérologie de l’Ouest, Site Gauducheau, Bvd J Monod, Saint-Herblain (Nantes), France

Background Information: Relevant animal models of human breast cancer are currently lacking, especially regarding the triple-negative breast cancer (TNBC) subtype, for which efficient therapies are needed. Recent studies indicate that spontaneous canine mammary carcinomas (CMCs) (which are common in France due to absence of early neutering) resemble human breast cancers, by pathology, tumor genetics, and biological behavior. However, the current molecular classification of human breast cancer has not been evaluated in canine samples yet.

Objective: To establish the prognostic value of the human immunophenotypic classification in dogs To evaluate CMCs as a model of human breast cancer including TNBC.

Methods: 350 CMCs treated by surgery alone were obtained from the Nantes Atlantic College of Veterinary Medicine, Food Science and Engineering (France) from 2005 to 2008. Recorded clinical data included breed, neutering, age at diagnosis, presence of metastases, cause and time to death. Histological records included the subtype of carcinoma, Elston & Ellis grade, presence of emboli and lymph node metastasis. By immunohistochemistry (IHC) using ER, PR, Her2 (Herceptest and Pathway® Her2), CK5/6, EGF-R and Ki67, CMCs were classified into the subtypes of human breast cancer according to Nielsen et al. IHC analyses were independently reviewed by four pathologists.

Results: The preliminary data in this abstract are based on 200 cases. The mean age at diagnosis was 10.8±2.1 years. 72.7% of dogs were intact female (27.3% of late neutering). 41% of dogs died due to
cancer progression (metastasis rate of 30%). The most common histologic subtype was simple tubulopapillary CMC (53%), then solid CMC (32%). The most common grades were grade II (49%) and III (43%). 53% of the tumors showed lymphatic emboli. 4 immunophenotypes were defined: luminal A (11.9%), luminal B (5.1%), basal-like (59.3%) and non basal-like (23.7%) triple negative CMCs. 8.8% of the CMCs were scored Her2 2+ but none were considered Her2-overexpressing as defined by a score of 3+; however, Her2 immunohistochemistry. 83% of CMCs were of the triple-negative subtype, associated with a shorter survival, as reported in human breast cancer. Infiltrative mammary cancer in dogs could be an interesting model for preclinical investigations. Final data based on 350 animals will be presented at the meeting.

PD08-11
Targeting Porcupine, a Critical Node for Wnt Signalling in Cancer.

Wnt ligands were first discovered based upon their transforming activity in the setting of mouse mammary tumor virus (MMTV) induced mammary carcinogenesis. Wnt signalling is required for normal development of the mammary gland and dysregulated Wnt signalling is implicated in cancers of the breast and colon among others. Furthermore, lobular breast cancer commonly shows reduced expression of E-cadherin that can lead to release of membrane-bound β-catenin into the cytoplasm and potentially increase Wnt signalling in the presence of Wnt ligand. To date, however, low-molecular weight therapeutic inhibitors of the Wnt pathway have not been developed. We set out to discover key points of intervention in the Wnt pathway and to develop low molecular weight inhibitors against such nodes. To this end, a cellular high-throughput screen for small molecule inhibitors of the Wnt pathway was performed. In this assay, cells secreting Wnt3a were co-cultured with cells harboring a luciferase reporter driven by the Wnt/beta-catenin responsive elements. Potent and specific Wnt pathway inhibitors were identified from this screen. To discern the mechanism of action of these compounds, a lead inhibitor was radiolabeled and used to probe protein targets in a radioligand binding assay. From these assays a candidate target of the inhibitor was identified as Porcupine. Porcupine is a membrane bound O-acetyltransferase enzyme that is required for and dedicated to palmitoylating Wnt ligands, a necessary step in the process of Wnt ligand secretion. Inhibition of Porcupine blocks Wnt dependent activities, including LRPs phosphorylation and the expression of Wnt target genes, such as Axin2, which in turn reduces the growth of cancer cells dependent on autocrine or paracrine Wnt signaling. A selective and orally bioavailable Porcupine inhibitor (NVP-LGK974) that inhibits the Wnt pathway in vitro and in vivo was developed through medicinal chemistry optimization. In preclinical evaluation, the compound robustly suppressed Wnt pathway signaling in vivo resulting in tumor regression in a murine breast cancer model driven by MMTV-Wnt1. In addition, this inhibitor attenuated tumor growth as a single agent and induced tumor regression in combination with Taxol in a human primary breast tumor model. Wnt signalling is also important for the maintenance and homeostasis of normal tissues including gastrointestinal tissue. However, preclinical rodent efficacy models of cancer demonstrate that intermittent pharmacological inhibition of Porcupine can be effective against the tumor and mostly spare the normal gastrointestinal tissue, with an acceptable therapeutic window.

A Phase I dose escalation study of NVP-LGK974 in patients with melanoma and lobular breast cancer to evaluate the safety, tolerability, PK, and PD properties is expected to begin this year.

PD09-01
Target-Based Therapeutic Matching in Early-Phase Clinical Trials in Patients with Advanced Breast Cancer and PIK3CA Mutations.
Janka F, Moulder SL, Wheeler JJ, Stepanek V, Falchook GS, Naing A, Hong DS, Fu S, Pika-Paul SA, Luthra R, Tsimeridou AM, Kurzrock R. The University of Texas MD Anderson Cancer Center, Houston, TX

Background: Therapeutic matching based on underlying molecular abnormalities showed promising results in early-phase clinical trials. PIK3CA mutations may predict response to therapies with PI3K/AKT/mTOR inhibitors.

Methods: Tumors from patients with breast cancer referred to the Clinical Center for Targeted Therapy (Phase I Program) were analyzed for PIK3CA mutations. Patients with PIK3CA mutations were treated, whenever feasible, with agents targeting the PI3K/AKT/mTOR pathway.

Results: Of 54 patients analyzed, 15 (28%) had PIK3CA mutations. PIK3CA mutations were found in 5/9 (56%) metastatic, 3/8 (38%) HER2-positive, 7/29 (24%) hormone receptor-positive, and 0/8 (0%) triple negative (excluding metastatic) breast cancers (P=0.07). Of the 15 patients with PIK3CA mutations, 12 (80%) were treated in clinical trials containing a PI3K/AKT/mTOR pathway inhibitor (median age, 54; median number of prior therapies, 3). Of these 12 patients, 3 (25%, 95% CI 0.09-0.53) had stable disease for more than 6 months (SD≥6 months; n=1) or a partial response (PR; n=2). Breast cancer patients without PIK3CA mutations treated on the same protocols had a rate of SD≥6 months/PR rate of 25% on 4 patients with a H1047R mutation treated with agents targeting the PI3K/AKT/mTOR pathway, 3 (75%) had SD≥6 months (n=1) or a PR (n=2) compared to 0 of 8 patients (0%) with other PIK3CA mutations (P=0.045). Patients with H1047R mutations had a median progression-free survival (PFS) of 8.5 months compared to 2 months in patients with other PIK3CA mutations (p=0.13).

Conclusion: Heavily pretreated patients with PIK3CA-mutant advanced breast cancer had a SD≥6 months/PR rate of 25% on protocols incorporating PI3K/AKT/mTOR inhibitors. Patients with mutation H1047R had significantly longer SD≥6 months/PR rate compared to those with other PIK3CA mutations. Our observations suggest that screening for PIK3CA mutations is warranted in larger numbers of patients with advanced breast cancer when treatment with PI3K/AKT/mTOR pathway inhibitors is planned.

PD09-02
Withdrawn by Author
PD09-03
Phase I/II Study of BKM120 in Combination with Trastuzumab in Patients with HER2 Overexpressing Metastatic Breast Cancer Resistant to Trastuzumab-Containing Therapy.
Saura C, Bendell J, Jerusalem G, Graña-Suárez B, Su S, Ru Q, De Buck S, Devisse C, Bosch A, Urruticoechea A, Beck JT, DiTomaso E, Rouyre N, Sternberg DW, Massacesi C, Hirawat S, Dirix L, Baselga J, Hospital Vall d’Hebron, Barcelona, Spain; Sarah Cannon Research Institute, Nashville, TN; C.H.U. Sart-Tilman, Liege, Belgium; Novartis Pharmaceuticals, Florham Park, NJ; Novartis Pharma AG, Basel, Switzerland; Hospital Clinico Universitario de Valencia, Valencia, Spain; Catalan Institute of Oncology, Barcelona, Spain; Highlands Oncology Group, Fayetteville, AZ; Novartis Institutes for BioMedical Research, Cambridge, MA; Novartis Oncology, Paris, France; Oncologisch Centrum AZ-St. Augustinus Oncology, Wilrijk, Belgium; Massachusetts General Hospital, Boston, MA

Introduction: HER2 amplification can activate the phosphatidylinositol-3-kinase (PI3K) pathway in breast cancer. Furthermore, trastuzumab (T) resistance can be associated with loss/deregulation of PTEN, which also causes activation of PI3K pathway signaling. BKM120, a potent orally bioavailable pan-class I PI3K inhibitor, could reverse resistance to T in vitro. Furthermore, administration of BKM120 with T demonstrated synergistic activity in preclinical models. The potential for combining BKM120 with T in heavily pretreated HER2+ metastatic breast cancer (MBC) patients (pts) will be determined in this study to substantiate the hypothesis for future development.

Methods: HER2+ MBC pts resistant to T-containing therapy (progression on or within 4 weeks since last T administration) received treatment in the dose-escalation portion of this multicenter trial. Prior anti-HER2-directed therapies were allowed. The primary objective was to determine the maximum tolerated dose (MTD) of BKM120 in combination with the standard dose of weekly T. A Bayesian logistic regression model with overdose control guided the dose escalation.

Results: Seventeen pts with MBC have been enrolled: 5 at 50 mg/day and 12 at 100 mg/day dose of BKM120. Escalation was conducted up to the MTD level of single agent BKM120, 100 mg/day. Patient characteristics: median age 46 years (range 34–70); most pts were heavily pretreated (reported range of prior chemotherapy lines 1–4). In preliminary results (cut-off date 1st June 2011), 7 pts discontinued treatment. BKM120 in combination with T showed a mean peak drug concentration and half-life similar to single-agent BKM120. T trough levels remained above the single agent efficacy threshold. Only one dose-limiting toxicity (G3 asthenia) was reported at the BKM120 dose of 100 mg/day. The MTD was determined to be 100 mg/day. Reported G3 adverse events were asthenia, altered mood, rash, GGT increase, hypokalemia, and hypersensitivity in 1 pt each. No G4 toxicity has been observed so far. With regards to activity, 2 partial responses (1 confirmed) and 3 occurrences of disease stabilization have been observed.

Conclusions: BKM120 in combination with T in heavily pretreated HER2+ MBC pts with T-resistance has an acceptable safety profile and has shown encouraging preliminary activity. The Phase II portion of the study is ongoing. An updated analysis of efficacy, the potential correlation with PI3K pathway alteration status overlaying HER2 activation, and pharmacodynamics study will be presented.

PD09-04
A Phase Ib, Open-Label, Dose-Escalation Study of the Safety and Pharmacology of the PI3-Kinase Inhibitor GDC-0941 in Combination with Paclitaxel and Bevacizumab in Patients with Locally Recurrent or Metastatic Breast Cancer.
Schiff F, P De Benedictis E, Gendream S, Gianni L, Krop IE, Levy G, Ware J, Wilders H, Winer EP. Catholic University, Leuven, Belgium; Istituto Nazionale dei Tumori, Milan, Italy; Genentech Inc., South San Francisco, CA; Dana-Farber Cancer Institute, Boston, MA

Background: GDC-0941 is a potent and selective oral pan-inhibitor of class I PI3K isoforms that demonstrates single-agent activity in xenograft models1,2,3. Increased phosphorylation of AKT has been observed in breast cancer (BC) cell lines treated with paclitaxel in vitro, suggesting dependence on the PI3K pathway for survival in response to chemotherapy treatment. GDC-0941 increases the antitumor activity of taxanes, associated with increased apoptotic cell death, in multiple BC xenograft models2.

Material and Methods: Patients (pts) with HER2-negative locally recurrent or metastatic BC (MBC) that received no more than 2 prior anti-cancer therapies for MBC (prior paclitaxel and/or bevacizumab permitted) were enrolled in a Phase Ib study (GDC4629g) of paclitaxel and GDC-0941 with and without bevacizumab using a 3+3 dose escalation design to evaluate the safety, tolerability, and PK and PD of the combination. Paclitaxel was given at 90 mg/m² on Days 1, 8 and 15 and bevacizumab, if applicable, at 10 mg/kg on Days 1 and 15 every 28 days. Two dosing schedules of GDC-0941 were examined: GDC-0941 given once-daily on Days 1-21 (“21+7” schedule) or given for 5 consecutive days followed by a 2-day drug holiday (“5+2” schedule), implemented to potentially improve the efficacy and safety of the combination treatment.

Results: We report data from 5 cohorts (25 pts). Sixteen of the 25 pts (64%) are hormone-receptor positive and 12 of 25 patients (48%) received prior treatment with a taxane, (all but one in the neo-adjuvant or adjuvant setting) and only one patient received prior treatment with bevacizumab. Pts in Cohort 1 received GDC-0941 60 mg given 21+7 with paclitaxel in Cycle 1; they were allowed to receive bevacizumab starting in Cycle 2. One DLT of Grade 3 subclavian vein thrombosis (in a pt with an indwelling catheter) was observed at this dose level. The cohort was expanded without any additional DLTs. In Cohorts 2 and 3, GDC-0941 was given 21+7 at 60 mg and 100 mg, respectively, with paclitaxel and bevacizumab starting in Cycle 1. One DLT of Grade 3 subclavian vein thrombosis (in a pt with an indwelling catheter) was observed at this dose level. The cohort was expanded without any additional DLTs. In Cohorts 2 and 3, GDC-0941 was given 21+7 at 60 mg and 100 mg, respectively, with paclitaxel and bevacizumab starting in Cycle 1. In Cohort 4, GDC-0941 was given 5+2 at 165 mg with paclitaxel only. Cohort 5, with 250 mg GDC-0941 given 5+2 with paclitaxel only, is currently under evaluation. The most common drug-related AEs in 22 treated patients are in Table 1. Preliminary PK for GDC-0941, paclitaxel and 6-hydroxy-paclitaxel were similar to historical profiles from previous studies of these molecules. One CR and 9 PRs (ORR 46%) have been observed to date.

Table 1: Drug-Related AEs Observed in >10% of Pts

<table>
<thead>
<tr>
<th>Preferred Term</th>
<th>ALL Grades</th>
<th>Grade 3/4</th>
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<tr>
<td>Diarrhea</td>
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<td>5</td>
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<tr>
<td>Alopecia</td>
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</tr>
<tr>
<td>Neutropenia</td>
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<td>36</td>
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<td>Peripheral neuropathy*</td>
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<td>9</td>
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<td>Epistaxis</td>
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<tr>
<td>Hypertension</td>
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<td>3</td>
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<tr>
<td>Anemia</td>
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<td>0</td>
</tr>
<tr>
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<td>5</td>
</tr>
<tr>
<td>Headache</td>
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</tr>
</tbody>
</table>
*includes term of peripheral sensory neuropathy

Cancer Res; 71(24 Suppl.) December 15, 2011 150s Cancer Research
Conclusions: GDC-0941 given 5+2 at doses up to 165 mg QD in combination with paclitaxel is well tolerated and dose escalation continues with encouraging clinical activity. Updated safety, PK and efficacy data will be presented.

1Juuttila et al, Cancer Cell 2009
2Yao et al., CCR 2009
3O’Brien et al., CCR 2010

PD09-05
SU2C Phase Ib Study of pan-P13K Inhibitor BKM120 Plus Aromatase Inhibitor Letrozole in ER+/HER2- Metastatic Breast Cancer (MBC).
Mayer IA, Balko JM, Kuba MG, Sanders ME, Yap J, Li Y, Winer E, Arteaga CL. Vanderbilt-Ingram Cancer Center, Nashville, TN; Dana Farber Cancer Institute, Boston, MA; MD Anderson Cancer Center, Houston, TX

Background: Experimental evidence and clinical data suggest that PIK3CA mutations (present in approximately 35% of ER+ cancers) are associated with de novo or acquired resistance to antiestrogen therapy. Considering that most breast cancers resistant to antiestrogen therapy retain ER and estrogen sensitivity, treatment of ER+ breast cancers with single-agent PI3K-targeted therapy may be insufficient to inhibit tumor growth.

Materials and Methods: To evaluate safety/tolerability, we initiated a phase Ib clinical trial of letrozole with the pan-P13K inhibitor BKM120 (Novartis) in post-menopausal patients with ER+/HER2– MBC. Letrozole (2.5 mg/d) and BKM120 (100 mg/d) were given on a 28-day cycle. When necessary, BKM120 was reduced to 80 and 60 mg/day. Treatment was continued until unacceptable toxicity or progression of disease. Disease was assessed every 2 months. FDG-PET imaging was performed at 2 weeks to identify changes indicative of pharmacodynamic modulation of PI3K/AKT.

Results: Twenty patients have been accrued; all but 2 patients had previously progressed on an aromatase inhibitor (AI) in the metastatic setting. Median age was 56 years; 85% of patients had metastatic bone disease, 70%, visceral metastases. Twelve patients are still on treatment, 2 of these are currently on BKM120 80 mg/day. Three patients discontinued treatment within 2 months of initiation due to grade 3 transaminitis, grade 2 irritability, and grade 2 hyperglycemia, despite dose reductions and/or optimal treatment. Most common toxicities are summarized in the table below. Transaminase elevation was the only DLT at 100 mg/d BKM120. Of the 12 evaluable patients that completed at least 8 weeks of treatment, 1 had a partial response, 7 had stable disease, and 4 had disease progression (RECIST). So far 3 patients have been on treatment for >4 months. Over 50% of patients had >25% reduction in their peak SUV at the 2-week FDG-PET scan.

Discussion: The letrozole + BKM120 combination is active in patients with ER+/HER2– MBC refractory to previous therapy with an AI. At this time, all patients’ primary diagnostic tumor biopsies and/or metastatic biopsies are being analyzed for the presence of PIK3CA, AKTI, or PTEN mutations and PTEN loss (immunohistochemistry). FDG-PET imaging at 2 weeks appears to be a useful pharmacodynamic biomarker of PI3K pathway inhibition.

Correlation of mutational analysis with clinical benefit will be presented at the meeting. Despite an overall safe side effect profile, an alternative administration schedule of BKM120 with letrozole is planned to enhance tolerability of the combination.

PD09-06
Phase II Trial of RAD001 (Everolimus), an mTOR Inhibitor, with Weekly Cisplatin and Paclitaxel in Patients with HER2-Negative Metastatic Breast Cancer (MBC).
Mayer IA, Means-Powell J, Abramson VG, Shyr Y, Balko JM, Kuba MG, Gharavi HM, Schlabach L, Arteaga CL, Pietenpol JA. Vanderbilt-Ingram Cancer Center; Nashville, TN; Vanderbilt-Ingram Cancer Center Affiliated Network (VICCAN)

Background: In basal-like breast cancers, drugs that either negatively modulate p63 and/or activate p73, such as cisplatin and paclitaxel, promote p73-dependent apoptosis. Further, the serine threonine kinase mTOR negatively regulates p73. Inhibition of mTOR with RAD001 upregulates p73, and results in p73-mediated apoptosis. Therefore, we conducted a phase II trial in patients with HER2-negative MBC to explore safety, tolerability and anti-tumor activity of the combination of paclitaxel, cisplatin and RAD001.

Materials and Methods: We initiated an open-label, phase II multi-institutional study of weekly cisplatin (25 mg/m²), paclitaxel (80 mg/m²) and daily RAD001 (5 mg), given on a 28-day cycle. Treatment was continued until unacceptable toxicity or progression of disease. All toxicities were documented using the NCICTCv.4. Disease was assessed every 2 months.

Results: A total of 55 patients were enrolled. Median age was 55 years; 62% of patients had prior chemotherapy with a median of 3 previous regimens in the metastatic setting. Sixty-three percent of patients had triple-negative disease, 81% patients had visceral disease, and 35% had bone metastases. Twenty-one patients are still on study. The most common toxicities are summarized in the table below. Neutropenia was the main cause for dose reductions, mainly after cycle 3. Only 1 patient developed febrile neutropenia. Of the 44 patients assessed for best response (RECIST) thus far, 11 had partial response, 21 stable disease, 9 disease progression, and 3 were not evaluable. Thirty-eight patients have discontinued treatment so far; 30 due to disease progression, 5 due to toxicity, and 3 withdrew consent. Current median time to progression on the evaluable patients is 6 months.

Discussion: The combination of RAD001, cisplatin and paclitaxel was overall very well tolerated despite cumulative pancytopenia. Significant antitumor activity in this heavily pre-treated patient population was seen. All patients’ primary diagnostic and/or metastatic tumor biopsies are currently being analyzed for the presence of PIK3CA and AKTI mutations, and immunohistochemical expression of p53, p63, p73, and PTEN. Microarrays are being generated to determine whether time to progression is superior in patients with basal-like breast cancers. Microarrays will be mined to identify a pretreatment profile that mirrors a low p63/high p73 gene expression signature. Correlation of mutational analysis and gene signatures with clinical benefit will be presented at the meeting.

www.aacrjournals.org 151s Cancer Res; 71(24 Suppl.) December 15, 2011
PD09-07
Withdrawn by Author

PD09-08
Combined Inhibition of mTORC1 with Temsirolimus and HER2 with Neratinib: A Phase I/II Study in Patients with Metastatic HER2-Amplified or Triple-Negative Breast Cancer.

Background: Hyperactivation of the PI3K-AKT-mTOR pathway is a postulated mechanism of resistance to anti-HER2 therapies and has also been described in triple-negative breast tumors. In HER2-amplified (HER2+) laboratory models, inhibition of this pathway induces activation of upstream receptor tyrosine kinases such as HER3. In triple-negative breast cancer (TN), HER1 overexpression has been identified and models show sensitivity to combined HER1 and mTOR inhibition. We hypothesize that dual inhibition of the PI3K pathway, HER1/2, and induced HER3 may be highly effective in patients with HER2+ or TN breast cancer (BC). This phase I/II trial is designed to determine the tolerability and possible efficacy of the mTOR inhibitor temsirolimus (T) plus the HER1/2 inhibitor neratinib (N) in patients with trastuzumab-refractory, HER2+ or TN BC. We will also explore mutational activation of the PI3K pathway in trastuzumab-refractory tumors as it relates to response to the T-N combination.

Methods: The phase I dose-escalation study evaluated T (flat dose IV weekly) plus N (240 mg oral daily) in patients with metastatic HER2+ or TN BC. Cycle length was 4 weeks. Phase I end points included definition of maximum tolerated dose (MTD) and response rate (RR) per RECIST. The phase II study has a Simon two-stage design and evaluates the HER2+ and TN patients separately. Phase II endpoints include progression free survival and duration of response. Response was evaluated radiographically every 8 weeks, toxicity assessed every 2 weeks. All patients underwent biopsy of metastatic disease for biomarker assessment. Activating mutations in PIK3CA were assayed using the Sequenom MassARRAY system. Expression of PTEN was assessed by immunohistochemistry utilizing a published scoring system.

Results: The phase I study enrolled 8 HER2+ patients who received a median of 5 (1-13) cycles of therapy. All patients had received trastuzumab and a median of 5.5 (2-12) prior lines of therapy. Frequent treatment-related grade 2 events were: hyperglycemia (4/8), elevated CPK (3/8), diarrhea (2/8), and rash (2/8). Grade 3 diarrhea was the dose-limiting toxicity. Other grade 3 toxicity was hyperglycemia (1/8); hematologic toxicities were not observed. The MTD of temsirolimus was neratinib is 8 mg IV weekly. Six patients treated at MTD were evaluable for response; 4 patients had PR, 1 had SD for a RR of 67%. PI3K pathway activation, through PIK3CA mutation activation or PTEN loss, was identified in 4/6 tumors analyzed and did not preclude response to temsirolimus and neratinib. Updated results reflecting the phase I/II patients will be reported.

Conclusions: Temsirolimus plus neratinib is active in trastuzumab-refractory HER2+ patients. The phase II study is ongoing and additional efficacy and safety data in both HER2-amplified and triple-negative breast cancer patients will be presented.

PD10-01
Prevalence of Dysfunctional Fanconi Anemia (FA) DNA Repair Pathway in Breast Cancer.
Ramaswamy B, Srividya V, Mullins DA, Carothers S, Young G, Wenrui D, Zhao W, Lustberg M, Leon M, Welslowski R, Layman R, Mrozek E, Shapiro CL, Villalona-Calero M. The Ohio State University, Columbus, OH; The Ohio State University

Purpose: BRCA1/2 deficient breast tumors are highly sensitive to poly-ADP-ribose polymerase inhibitors (PARPi). The Fanconi Anemia (FA) associated gene products along with BRCA 1/2 function in a common pathway that regulates the cellular response to DNA damage, suggesting that tumors with dysfunction of any of the components of FA network would be susceptible to PARPi. Understanding the prevalence of such defects in breast tumors using reproducible methodology will help us target these tumors with novel agents and potentially improve outcomes. Hence we sought to assess the prevalence of FA pathway defect in breast tumors by the absence of nuclear FANCD2 (a pivotal protein in the FA/BRCA pathway which is monoubiquitylated in the nucleus in response to DNA damage) repair foci using a novel immunofluorescence method and correlate this with known molecular markers of breast cancer.

Methods: Using primary tumors obtained from the ongoing PARPi clinical trials (NCT01017640 and NCT01251874) and tumor bank, we evaluated 102 breast tumors for the somatic functionality of the FA pathway (FANCD2 foci formation) by the FA Triple Stain Immunofluorescence (FATSI) test performed in a CLIA-certified laboratory using paraffin embedded tissues. The tissue sections are incubated with a primary antibody cocktail of rabbit polyclonal FANCD2 antibody and a monoclonal anti-Ki67 mouse antibody, followed by co-incubation with a secondary antibody (FITC conjugated to anti-rabbit IgG and Alexafluor 594 donkey anti-mouse), mounted on glass slides in a DAPI containing embedding medium and evaluated by a fluorescence microscope. Absence of nuclear FANCD2 formation in 100 proliferating tumor cells was considered positive for FA defect. Hormone receptor (HR) and Her2 status was compared between the groups using Fisher’s exact test.

Results: A total of 102 primary breast tumors were analyzed for FANCD2 by FATSI test of which 62 were triple negative (TN), 37 were HR positive and 3 were Her2 positive. Of these, 29 tumors (28%) were positive for FA defect with no significant differences among the molecular subtypes (26% in TN vs 32% in HR + vs 33% in Her2+).

Table 1

<table>
<thead>
<tr>
<th>Factor</th>
<th>FA defect = no</th>
<th>FA defect = yes</th>
<th>p-value</th>
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<tr>
<td>ER Pos</td>
<td>20 (64.5%)</td>
<td>11 (35.5%)</td>
<td>0.84</td>
</tr>
<tr>
<td>PR Pos</td>
<td>21 (74.1%)</td>
<td>7 (25.9%)</td>
<td>0.81</td>
</tr>
<tr>
<td>HER2 3+</td>
<td>16 (66.7%)</td>
<td>7 (33.3%)</td>
<td>0.30</td>
</tr>
<tr>
<td>TN</td>
<td>46 (74.2%)</td>
<td>16 (25.8%)</td>
<td>0.51</td>
</tr>
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</table>

Conclusions: We report a novel methodology to efficiently screen archival FFPE tumors for somatic functional defect of FA DNA repair pathway and demonstrated a high prevalence (one-third) in breast tumors irrespective of molecular subtype. We are currently conducting clinical trials with PARPi including patients with tumors that test positive for FA defect to demonstrate if such tumors are sensitive to PARPi.

PD10-02
Sporadic Breast Cancers Show Defects in the BRCA1-BRCA2 Pathway of Homologous Recombination in All Biomarker-Defined Sub-Types of Breast Cancer.
Powell SN, Matter RW, Deliste R, Bindra R, King T, Giri D, Park J. Memorial Sloan-Kettering Cancer Center, New York, NY

Background: Mutation carriers of BRCA1 and BRCA2 are well known to develop early onset breast cancer, with loss of the second
allele occurring in the development of the tumor. However, by array comparative genomic hybridization (aCGH) studies, some sporadic breast cancers have a similar “genetic landscape” as BRCA-mutation carriers, showing large losses and gains across the genome. We have now identified that DNA repair defects involving the BRCA1-BRCA2 pathway can occur in the absence of mutations in either gene and in the absence of a deficiency in either protein.

Methods: Fresh human breast cancer samples were irradiated, ex-vivo, to look for the ability to assemble RAD51 protein macro-complexes or foci. Primary breast cancer specimens were obtained from consented patients with non-metastatic, invasive carcinomas following lumpectomy or mastectomy, without neoadjuvant cytotoxic or hormonal therapy. A single cell suspension was prepared from the tumor, with one half irradiated to 10Gy and the other half mock-treated. After 4h, cells were mounted, fixed on slides, and stained with anti-Rad51, anti-BRCA1, and anti-γH2AX antibodies. At least 200 nuclei were examined and scored using confocal microscopy. A failure to induce RAD51 nuclear foci by 2-fold after ionizing radiation was designated as defective in homologous recombination (HR).

Results: For the 71 patient samples analyzed, we have 14 triple-negative tumors, of which 6 are HR-defective (42.8%); for Her2-amplified tumors, we have 6/19 (31.6%) that are HR-defective and for ER+ tumors 6/38 (15.8%). The overall incidence of HR-defective tumors is 18/71 (25.3%), which is substantially higher than we would have expected.

Incidence of BRCA-HR pathway defects by Biomarker Group

<table>
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<tr>
<th>DNA Repair Status</th>
<th>BRCA1 Expression</th>
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<tr>
<td>HR-defective</td>
<td>6</td>
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<td>HR-proficient</td>
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<td>Her2-amp</td>
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</tbody>
</table>

Known mutation carriers were not included in the study, since these samples are BRCA-HR-defective in all cases we have tested. For the more recently acquired samples, we have undertaken additional tests to characterize the tumors: short-term growth assays in response to mitomycinC to validate that HR-defective tumors are indeed sensitive to cross-linking agents; and, a pilot analysis to study aCGH patterns in HR-defective tumors. The latter studies have compared 6 repair-deficient and 6 repair-proficient tumors using unsupervised cluster analysis of large block deletions or large block copy number increase, which clearly reveal that large block alterations are linked to repair-deficient tumors.

Conclusions: There is a significant incidence of BRCA-HR defective sporadic breast cancers, as determined by RAD51 function in response to ionizing radiation plus genetic landscape alterations using aCGH. The pool of breast cancers that are susceptible to repair targeting strategies is larger than expected and is not readily defined by conventional diagnostic biomarker classification. These findings may account for the failure of the recent phase III study of the addition of iniparib to carboplatin and gemcitabine in triple-negative cancer as only a minority of the tumors will be susceptible to this targeting strategy.

PD10-04
Exploration of the Relationship between Loss of PTEN and BRCA1 Expression in Triple Negative Breast Carcinoma.


Based on the mechanism of action of PARP inhibitors, BRCA1ess breast and ovarian cancers could be defined as the optimal group of tumours to target with such drugs. As the BRCA1ess phenotype is not fully defined at present, a surrogate phenotype of triple negative breast cancer (TNBC) has been proposed. Results of recent clinical trials involving PARP inhibitors in the context of TNBC do not demonstrate a significant improvement in overall survival or progression free survival, partly due to the inaccurate selection of certain tumours. Data from cell culture studies suggests that loss of PTEN expression could be a marker of PARP inhibitor sensitivity.

Materials and Methods
A retrospective study of 160 TNBC resected at Institut Bergonie between 1989 and 2010 was undertaken. Inclusion criteria were: ER & PR immunohistochemical (IHC) negativity (<10% tumour cells positive), non-amplification of HER2 (IHC score 0, 1, or 2 with negative FISH analysis) and an invasive ductal carcinoma resected before chemotherapy. For some familial cases, germline BRCA1 mutation screening had been performed in a diagnostic setting and was accompanied by genetic counselling. PTEN IHC, point mutation and gross rearrangement screening, along with BRCA1 expression and promotor methylation analysis (through real-time PCR and bisulphite treatment, respectively) and 53BP1 IHC were undertaken on this series of TNBC.

Results
Twenty percent of sporadic and familial TNBC demonstrated reduced BRCA1 expression with a greater than 7 fold reduction in expression. Almost half of tumours demonstrated a complete loss of PTEN expression as assessed by IHC, with a deleterious PTEN point mutation identified in 5% of cases. Although a slight correlation is observed between the complete loss of PTEN and a reduction in BRCA1 expression, a complete overlap of these two alterations was not observed; tumours with reduced BRCA1 expression do not always demonstrate complete negativity on PTEN IHC and certain tumours displaying complete negativity on PTEN IHC do not show a reduction in BRCA1 expression. Full immunohistochemical characterization of the tumour series (ER, PR, HER2, AR, CK5/6 & EGFR) together with the results of PTEN, BRCA1 and 53BP1 analyses will be presented.

Conclusion
These results suggest that at present, loss of PTEN expression is not a suitable surrogate marker for tumours with reduced BRCA1 expression. This should be borne in mind for ancillary studies of clinical trials involving PARP inhibitors in the treatment of breast tumours and further investigation is warranted.

PD10-03
Withdrawn by Author
Identification of Novel BRCA1 Transcriptional Targets That Promote the Survival of BRCA1-Mutated Estrogen Receptor-α Negative (ER-ve) Breast Tumours.

Lamers E, Haddock P, Cochrane DJ, Gorski JJ, Blayney J, McDyer FA, Mulligan JM, Mullan PB, Couch FJ, Kennedy RD, Harkin PD, Quinn JE. Queen’s University of Belfast, Belfast, Antrim, United Kingdom; Almac Ltd., Craigavon, United Kingdom; Mayo Clinic College of Medicine, Rochester, MN.

Background

The BRCA1 tumour suppressor gene is mutated in the germline of women who are predisposed to developing breast cancer. Gene expression profiling has identified at least five different breast cancer subtypes with BRCA1-mutated breast tumours clustering with triple negative breast cancers. The majority of BRCA1-mutated breast tumours are characterised as being negative for the estrogen receptor-α (ER-α), however, the underlying molecular biology of these tumours has not yet been fully determined. The aim of this study is to identify and characterise novel proliferation-associated BRCA1 target genes that are activated in BRCA1 mutated estrogen receptor negative (ER-ve) breast tumours.

Methods

Gene expression profiling and data analysis was performed on a cohort of 46 FFPE (Formalin Fixed Paraffin Embedded) derived BRCA1 mutated (ER-ve) breast tumours and matched sporadic controls using the Almac Diagnostics Breast DSA research tool. Profiling was also performed on a panel of 15 breast cancer cell lines. Bioinformatics analysis was performed using Oncomine, DAVID and Metacore. High throughput siRNA screening using HiPerFect were performed on the Qiagen Flexiplate siRNA. Validation of gene targets was performed by qRT-PCR and ChIP assay. Clonogenic assays were performed to independently validate the effect of selected target genes on cell survival.

Results

A list of differentially expressed transcripts was derived from the comparison of 23 (ER-ve) BRCA1 mutated breast tumours and 23 matched sporadic controls. A genome-wide ChIP-Chip promoter analysis was also performed in MCF7 breast cancer cells. By overlapping these two datasets, a list of tumour derived BRCA1 promoter bound target genes was identified. Functional analysis of this gene list has identified the main pathways and processes that are deregulated in BRCA1 mutated (ER-ve) breast cancer including: (1) immune response (2) induction of the epithelial to mesenchymal transition (EMT) (3) cell cycle regulation and (4) apoptosis and survival. Hierarchical clustering of these 46 breast tumours and 15 breast cancer cell lines was performed and the BRCA1 mutated (ER-ve) breast cancer cell lines (MDA436 and SUM149) were identified as those that best reflect the biology BRCA1 mutated (ER-ve) tumours. High throughput siRNA screening in these cell lines has identified a set of transcripts that when inhibited have a negative impact on cellular proliferation. Independent validation by qRT-PCR, ChIP assay, western blotting and clonogenic assays have confirmed HE4 (WFDC2) as a novel BRCA1 target gene that provides a growth advantage in BRCA1 mutated (ER-ve) breast cancer cells.

Conclusions

Gene expression profiling of an extensive cohort of BRCA1 mutated (ER-ve) breast tumours and matched sporadic controls has identified a profile of BRCA1 mutated (ER-ve) breast cancer. This list has been further refined to generate a list of BRCA1 promoter bound transcriptional target genes. High throughput siRNA screening has revealed a panel of genes that are implicated in the proliferation of BRCA1 mutated (ER-ve) breast cancer. HE4 has been identified as a novel BRCA1 transcriptional target gene that promotes the survival of BRCA1 mutated (ER-ve) breast tumours.

Loss of the Retinoblastoma Tumor Suppressor (RB) in Triple Negative Breast Cancer Is Associated with a Favorable Prognosis.

Witkiewicz AK, Kline J, Mitchell E, Ertel A, Knudsen ES, Thomas Jefferson University, Philadelphia, PA; Kimmel Cancer Center, Philadelphia, PA

Introduction

The retinoblastoma tumor suppressor gene (RB), initially identified in the pediatric tumor retinoblastoma, has been shown to be functionally inactivated in a variety of other tumor types. RB functions as a key regulator of cell cycle progression and modulates the response to a variety of physiological and clinically relevant stresses. In breast cancer, loss of RB has been shown to have prognostic significance and influence therapeutic response; however, a comprehensive analyses of RB in triple negative breast cancer (TNBC) has not been performed. Although TNBC represents only 15-20% of breast cancers it accounts for approximately half of breast cancer deaths. A subset of patients with TNBC will have a dramatic and durable response to standard chemotherapy. However, currently there are no markers that identify this patient subset. Since RB loss impinges on the response to chemotherapy, the goal of this study was to investigate the impact of RB-status on clinical management of TNBC.

Material and Methods

A cohort of 220 patients diagnosed and treated at Thomas Jefferson University Hospital was included in the study. RB-status was evaluated by immunohistochemistry (IHC) using 3 markers: p16ink4a (MTM Laboratories, Cat #: 9518,1:50 dilution ), Ki67 (AbCam, Cat #: ab16667, 1:600 dilution) and RB (Thermoscientific, Cat #: MS-107-B, 1:50 dilution) through automated image analysis (Aperio). Cases were considered negative for RB when no neoplastic cell nuclei demonstrated labeling in sections in which stromal cells and endothelial cells stained; Ki67 and p16ink4 were scored using established published criteria. Gene expression profiling was performed on 12 cases (6 RB positive and 6 RB negative by IHC) and RB pathway status in these samples was evaluated using an RB-loss signature developed by our group. Parallel analyses of TNBC expression data sets were also employed to define the relationship of the RB pathway with clinical outcome. Overall survival was analyzed using LogRank test and Cox proportional hazard model. Data were analyzed in SAS 9.1 (SAS Institute Inc., Cary, NC, USA).

Results

176 patients had samples that could be scored for RB, p16ink4 and Ki67 expression. 46% (71/176) showed RB loss. RB loss was associated with high p16ink4a expression and high Ki67 proliferation index in 93% (66/71) of samples and correlated with RB loss signature in all but one sample. RB loss and high p16ink4 expression was associated with a longer overall survival in this cohort (p=0.003). In contrast, patients with TNBC showing RB positivity and high p16ink4 had the worse survival (median survival 4 months, 10 year survival 23%). These findings were consistent with gene expression profiling data that indicate specific loss of RB function in TNBC is associated with improved response to chemotherapy and improved survival.

Conclusion

RB loss appears to be a predictor of a favorable clinical outcome in TNBC treated with conventional adjuvant chemotherapy.
PD10-07
microRNA Profiles of Breast Tumors Identifies the miR 17-92 Cluster as a Group of Potentially Essential Oncomirs in Triple-Negative Breast Cancer.
Son BH, Birkbak NJ, Tian R, Iglehart D, Wang ZC, Richardson AL. Dana-Farber Cancer Institute, Boston, MA; Asan Medical Center, Seoul, Korea; Technical University of Denmark, Lyngby, Denmark; Brigham and Women’s Hospital, Harvard Medical School, Boston, MA

Introduction: MicroRNAs (miRNAs) are a class of short non-coding RNAs of about 20-24 nucleotides that act post-transcriptionally to modulate gene expression by sequence-specific base-pairing with target miRNAs. While the function of human miRNA remains largely unknown, certain miRNAs have previously been implicated in cancer development and progression. In this study, we investigate the expression of 734 miRNAs across a cohort of 7 normal breast samples and 104 breast cancer tumors including 70 ER-/PR-/HER2-(triple negative, TN), 15 ER+/PR+, and 19 HER2+.

Methods: We used the novel Nanostring technology to quantify the expression of 734 miRNAs. Unsupervised hierarchical clustering was performed using miRNAs that were detected as expressed in at least one sample. A two-sided Wilcoxon rank sum test was used to identify miRNAs with a significantly differential expression between two sets of samples.

Results: In agreement with previously published results, we found that tumor samples clustered into the luminal and TN subtypes using unsupervised hierarchical clustering on the expressed miRNAs. Interestingly, microRNA expression was similar between HER2 amplified ER+ and HER2 amplified ER-, suggesting a common luminal origin. When we specifically compared luminal (ER+ or HER2+) to TNBC, we found that 50 miRNAs showed significant differential expression. In particular, the miR-17-92 cluster(1) was expressed at significantly higher levels in TN tumors. Within the luminal tumors the miR-17-92 cluster was strongly correlated with proliferation and grade; although miR17-92 was expressed at high level, there was no correlation with proliferation in the TN tumors. miR17-92, which inhibits translation of E2F1(1), is in a regulatory network with myc and E2F to control the balance between proliferation and apoptosis(2). The absence of correlation between miR17-92 and proliferation in the TN tumors suggests disruption of this regulatory network. Indeed, we found a positive correlation between expression of miR17-92 and p16 and inverse correlation with RB across the tumor set. The high p16/low RB expression in TN tumors is consistent with disruption of the RB pathway (3) and suggest these TN tumors may depend on miR17-92 to down-modulate expression of E2F1 which would otherwise induce apoptosis.

Conclusions: Our results show that miRNA expression in breast cancer tumors differs depending on subtype. We also find evidence to suggest subtype-specific miRNA regulation of proliferation and a particular dependency of TN tumors on the miR17-92 cluster for survival.


P1-01-01
A Rat Monoclonal Antibody Against Bone Sialoprotein II Is Active in Preventing and Treating Tumor Growth and Osteolytic Lesions in Nude Rats Induced by MDA-MB-231 Breast Cancer Cells. Berger MR, Zepp M, Armbruster FP. DKFZ, Heidelberg, Germany; Immunndiagnostik Comp., Bensheim, Germany

The SBLING protein bone sialoprotein II (BSPII) has been found implicated in lytic skeletal metastasis. Previous experiments had shown that targeting BSPII by a polyclonal IgY antibody or knock down of the gene’s transcript are instrumental in inhibiting experimental lytic skeletal metastasis. The aim of this study was to investigate the preventive and therapeutic effects of a new rat monoclonal anti BSPII antibody against tumor growth and osteolytic activity of MDA-MB-231 breast cancer cells growing in nude rats. In the preventive arm of this study, rats were pre-treated with the antibody (0 and 10 mg/kg) starting one week before tumor inoculation. In parallel, MDA-MB-231™ cells were incubated with the antibody for one week (0 and 0.5 mg/ml). Following inoculation of 1x10⁶ MDA-MB-231™ breast cancer cells into the femoral artery of 6 male nude rats, respectively, lesions were expected to develop only in the tibia, femur or fibula of the respective hind leg. Their appearance and development were monitored for six weeks by light emission, caused by luciferase mediated metabolism of luciferin. Photon emission was recorded at regular intervals by a Xenogen IVIS 100 imaging system. In the treatment arm of this study, the antibody administration (10 mg/kg/week) started when tumor bearing rats had shown stable tumor growth. Experimental groups of rats received the first treatment either at four (late onset; n = 8) or at two (early onset; n = 6) weeks after tumor cell implantation. Tumor bearing animals were (sham-) treated and followed for up to 8 weeks.

All 6 control rats of the preventive arm showed steady tumor growth. In variance, the rats receiving MDA-MB-231™ cells that had been pre-exposed to the antibody and those rats, which had been pre-treated with the antibody showed clearly reduced light emission as indicator of reduced tumor growth. At 6 weeks after tumor cell inoculation, only 1 of 6 rats was positive for light emission in the group receiving pre-exposed tumor cells (p<0.01), 3 of 6 rats were positive in the group receiving pre-treatment with the antibody (p<0.05), and 1 of 6 rats was positive in the group receiving both, pre-exposed tumor cells as well as pre-treatment with the antibody (p<0.01).

In the treatment arm, all but one untreated tumor bearing rats showed rapid tumor growth accompanied with lytic destruction of femur and tibia of the respective hind leg (18/19; tumor take rate 95%). In contrast, rats treated with the anti-BSP antibody did not show a significant increase in light emission nor a clinical deterioration. In fact, 6 of 8 rats receiving the late onset therapy didn’t show any light emission after 4 to 6 weeks (p = 0.01 versus control) as well as 4 of 6 rats receiving the early onset therapy with the antibody (p < 0.05). Radiological and histological examination confirmed that animals without light emission were free of tumor growth, corresponding to a complete remission.

In conclusion, the rat monoclonal antibody directed against BSP II is a powerful tool in treating experimental skeletal metastasis and warrants further development.
PI-01-02
T Cell Is a Key Player in the Establishment of Cancer Associated Pre-Metastatic Bone Disease.
Bonomo A, Monteiro AC, Leal AC, Alves AP, Frusciante T, Braun S, Azevedo RB. Instituto Nacional de Cancer; Rio de Janeiro, Brazil; Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil; Universidade de Brasilia, Brasilia, Brazil

Syngeneic mouse models of malignant diseases have the important advantage of allowing studies to be carried out in immunocompetent animals. In this work, the 4T1(metastatic) and 67NR (non-metastatic) sibling cell lines of mammary mouse carcinomas, syngeneic to BALB/c mouse were used to study tumor specific immune response and its impact on bone disease. By day 11 after tumor injection, in the absence of metastasis, pro-osteoclastogenic cytokines IL17F, TNFα, IL-1β and RANKL were present in the BM of 4T1+ mice in contrast to the virtual absence of these in the BM of 67NR+ animals. More important, imaging studies (histomorphometry and microCT) showed that trabecular bone mass is close to maximum loss (around 50%) already by day 11, in the absence of metastasis. These results support the possibility of T cell involvement in pre-metastatic lesion. Indeed, in vitro, BM T cells from 4T1+ mice, but not from 67NR+, could induce OC differentiation in response to tumor antigens ascertained by TRAP enzymatic activity, morphology and osteolytic disk assay. In vivo transfer experiments showed that T cells from 4T1+ mice (Day 11), when transferred into nude mice produce 3 times more IL17F and 4 times more RANKL than donor 67NR+ T cells. Moreover, trabecular bone at day 14 after transfer of 4T1+ T cells was already 30% that of normal nude mice in contrast to 100% from 67NR+ T cells. Of note is the fact that these activities are achieved in the absence of the metastatic 4T1 tumor in the nude host, but challenged with either 67NR or protein antigen extract. To address the role of T cell derived IL17F and RANKL, T cells from 4T1 bearing mice (day 11) were silenced with shRNA specific for each of these cytokines and transferred into nude mice. Animals were challenged with soluble tumor antigen. Silencing IL17F had no effect over bone loss. However, RANKL silencing completely inhibited osteolytic lesions. Together, these results indicate that RANKL in T cells mediate the osteolytic lesions and that cancer induced bone disease can be metastasis independent. Since osteolytic lesions are believed to be important to feed the tumor with growth factors in a vicious cycle established in the metastatic niche, we asked if T cells could be preparing the pre-metastatic niche. When 4T1 cells are injected into athymic nude mice, bone metastasis is not observed up to day 24, while by day 20 it is already present in euthymic BALB/c animals, indicating that T cells are critical to prepare the seeding soil for tumor cells within the bone. We are currently investigating the role of T cells in the establishment of metastasis at later time points as well as in the primary tumor growth. Our results strongly suggest that tumor cells, according to its metastatic activity, can modulate T cell activity systemically. Moreover, and not less important, we show that cancer induced bone disease starts before metastatic colonization and is mediated by RANKL expressed in tumor specific T cells. We propose that this is the very first step on the establishment of the “vicious cycle” which will allow tumor growth. We believe this work can open new avenues on the prognostic evaluation and treatment of women with breast cancer.

PI-01-03
The Cytoxicity of Select Neutrophils in Cancer Patients and the Role of Chemokines in Inducing Neutrophil Cytoxicity.
Comen E, Granot Z, Norton L, Benaza R. Memorial Sloan-Kettering Cancer Center, New York, NY; Memorial Sloan-Kettering Institute, New York, NY

Background: Using murine mammary tumor models, recent research conducted by our laboratory at the Sloan-Kettering Institute indicates that select neutrophils are mobilized by a primary breast tumor and uniquely have the capacity to inhibit metastatic seeding in the lung (Granot Z et al. unpublished). We sought to evaluate the cytotoxic role of select neutrophils in the peripheral blood of breast cancer patients as contrasted with those from women without breast cancer and women with DCIS. In addition, we sought to determine whether the addition of select chemokines to neutrophils from a DCIS patient could induce cytotoxicity.

Methods: Neutrophils were purified from the blood of 21 newly diagnosed pre-operative breast cancer patients without evidence of metastatic disease, 9 healthy female volunteers with no history of any cancer, and 3 patients with newly diagnosed DCIS. Cytotoxicity was evaluated by incubating isolated neutrophils with luciferase labeled MDA-MB-231 cells. Luciferase activity, as a reflection of % cell kill, was measured using a Bio-Tek microplate luminescence reader. Neutrophils from a DCIS patient with low cytotoxicity were then co-cultured with various CC chemokines (CCL2, CCL3 and CCL5) or CXCL chemokines (CXCL1, CXCL12, and CXCL16) at 100ng/ml.

Results: Significant cytotoxicity was notably observed when MDA-MB-231 cells were co-cultured with neutrophils purified from patients with invasive tumors. Pre-operative breast cancer patients (n=21) had a cell kill range of 0-30% (mean = 12.1%), whereas healthy subjects (n=9) had a cell kill range of 0.2-8% (mean = 2.6%), p<0.004. DCIS patients (N=3) had a cell kill range of 3-4% (mean = 2.7%). The addition of select chemokines to neutrophils from a DCIS patient with low cytotoxicity (3.2%) resulted in significant increases in cytotoxicity. Table 1 indicates the relative cytotoxicity percentages from the addition of each chemokine.

Conclusions: To date, this preliminary work is the first to demonstrate the cytotoxic role of select neutrophils in the peripheral blood of breast cancer patients as contrasted with those from women without breast cancer. We further demonstrate the novel induction of neutrophil cytotoxicity by select chemokines. Further studies are needed to evaluate the prognostic and therapeutic role of cytotoxic neutrophils as well as the role of chemokines in neutrophil cytotoxicity.

Table 1: Induction of neutrophil cytotoxicity by select chemokines

<table>
<thead>
<tr>
<th>No chemokines added</th>
<th>CCL2</th>
<th>CCL5</th>
<th>Mip1a</th>
<th>CXCL1</th>
<th>CXCL12</th>
<th>CXCL16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytotoxicity</td>
<td>3.2%</td>
<td>20%</td>
<td>29%</td>
<td>37%</td>
<td>10%</td>
<td>49%</td>
</tr>
</tbody>
</table>

PI-01-04
Immunological Effects of Bisphosphonates on γδT Cells in Breast Cancer.
Sugie T, Tanaka Y, Toi M, Minato N. Graduate School of Medicine, Kyoto University, Kyoto, Japan

Background: Human γδT cells play an important role in the first-line of defence. Accumulating data demonstrate that γδ T cells recognize nonpeptide antigens such as IPP and HMBPP and activated γδT cells have potent cytotoxic activity against tumor cells. Nitrogen-containing bisphosphonates like zoledronic acid inhibit farnesyl diphosphate synthetase, resulting in the accumulation of upstream metabolites such as IPP. Previous studies on the adoptive transfer of ex-vivo amplified γδ T cells demonstrated the safety and feasibility of γδT cell-based immunotherapy for metastatic cancer. According
to ABCSG-12 clinical trial, zolenderacic acid improved DFS of patients with early-stage breast cancer under adjuvant endocrine therapy. Whereas a precise mechanism remained unclear, it is likely that γδT cells are involved in this anti-tumor effect in breast cancer.

**Materials and Methods:** Peripheral blood mononuclear cells derived from 63 (54 untreated non-metastatic and 9 metastatic) patients with breast cancer were examined for the baseline population of γδT cells and their proliferative responses to zolenderacic acid in vitro. Tumor-infiltrating lymphocytes were isolated from 20 tumor biopsies and 2 pleural effusions. Peripheral blood γδT cells were stimulated with 5 μM zolenderacic acid and cultured in the presence of 100 IU/ml IL-2 for 10 days. Amplified γδ T cells were analyzed for cytokine production and cytotoxic activity.

**Results:** The proportion of γδ (TCRVδ2-bearing) T cells among peripheral blood CD3+ cells was 4.2 ± 5.0%. Although it was equivalent to the previously reported value of 4.3 ± 2.2% in healthy volunteers, 14% (9/63) of patients exhibited an increase in the number of peripheral blood γδT cells at baseline. The level of TCRVδ2 T cells with potent anti-tumor activity was significantly higher than that of TCRVδ1 T cells (4.2± 5.0% vs. 1.5 ± 1.8%, p<0.001), whereas they were present in tumor-infiltrating cells at essentially the same levels (4.4± 9.6% vs. 3.7 ± 9.7%, p=0.434). Percentage of TCRV δ2+ CD3+ cells among amplified γδ T cells in vitro and cytokine (IFN-γ and TNF-α) production by them were significantly proportional to the initial level of TCRVδ2/CD3+ ratio in peripheral blood. Upon treatment with zolenderacic acid, breast cancer cell lines became susceptible to γδ T cells.

**Conclusions:** γδ (TCR Vδ2-bearing) T cells increased in some patients with breast cancer and the TCRVδ2/CD3+ ratio at baseline was correlated with the efficiency of proliferation and cytotoxic activity when treated with zolenderacic acid. Zolenderacic acid efficiently sensitized breast tumor cells to be lyzed by γδ T cells. The present results suggest that γδ T cells stimulated with zolenderacic acid can be utilized for immunotherapy against breast cancer.

**P1-01-05**

**Conditioning by the Tumor Environment Turns Invariant Natural Killer T Cells into Negative Regulators of Anti-Tumor Immunity Elicited by Treatment.**

Pilones KA, Demaria S. NYU School of Medicine, New York, NY

Background: Local suppression in the tumor microenvironment remains an important obstacle to success of immune-based therapy. We have previously shown in the 4T1 model of metastatic breast cancer that combination radiotherapy (RT) to the primary tumor and CTLA-4 blockade can elicit a robust anti-tumor effector response that can inhibit metastases and prolong survival. However, complete cure and long-term survival may only be seen in a small fraction of mice, prompting us to investigate new ways to improve the response elicited by treatment. InNKT cells are known to be powerful regulators of tumor immunity as a result of their ability to rapidly secrete a broad range of cytokines, but the mechanisms regulating their switch from a stimulatory to a regulatory role remain poorly defined. We have previously shown that InNKT cells negatively regulate the response to treatment with RT and CTLA-4 blockade in 4T1 tumor-bearing mice (Clin Cancer Res 15:597, 2009). Here, we test the novel hypothesis that conditioning of InNKT cells to perform this regulatory role occurs within the tumor microenvironment. Specifically, we address whether 4T1 tumor cells, directly or indirectly, promote inNKT cell regulatory phenotype. Methods: For in vitro experiments, 4T1 tumor or LPS-matured bone marrow-derived dendritic cells (BMDC) were loaded with α-galactosylceramide and used to stimulate the NKT DN32.D3 hybridoma cells. In some wells, BMDCs were supplemented with 4T1 conditioned media or loaded with tumor lysates from 4T1 cells. For in vivo experiments, wild-type and InNKT-deficient mice were inoculated s.c with 4T1 cells for enumeration of DCs on immunostained sections.

**Phenotypic profile of DCs within tumor and draining lymph nodes were assessed by flow cytometry. Results: 4T1 cells are positive for CD1d by real-time RT-PCR; surface CD1d were barely detectable by flow cytometry but sufficient to induce activation of DN32.D3 cells when irradiated 4T1 cells were loaded with α-GalCer. BMDC supplemented with conditioned media from untreated or irradiated 4T1 cells, or loaded with tumor lysates from irradiated 4T1 cells were also able to activate DN32.D3 cells, and this effect was partially inhibited by blocking CD1d mAb (1B1) in a dose-dependent manner. In vivo, we found that inNKT-deficient mice showed significantly (p<0.04) higher densities of tumor infiltrating DCs than wild type mice. Moreover, DC in tumor and tumor-draining lymph nodes expressed markers of maturation in the absence but not presence of InNKT cells. Conclusion: Results indicate that a 4T1 derived factor, possibly a lipid antigen that can be presented directly by tumor cells themselves or indirectly by BMDCs, induces activation of InNKT cells in vitro. In vivo, data suggests that inNKT cells downregulate the response to treatment by controlling the population of DC present in the tumor and draining lymph nodes. Since DC are essential for cross-presentation of the tumor antigens released by RT-induced cell death, reduced DC number may impair the activation of anti-tumor T cells. Supported by DOD Postdoctoral Award W81XWH-10-1-1205.

**P1-01-06**

**Mechanisms of Tumor Immune Escape in Triplenegative Breast Cancers (TNBC) with and without Mutated BRCA 1.**

Segerer SE, Kapp M, Hahne JC, Dietl J, Engel J. Medical University of Würzburg, Würzburg, Germany

**Background**

Triplenegative breast cancer (TNBC) is associated with a dismal prognosis, although these tumors are chemo-sensitive. This phenomenon could be at least in part due to tumor immune escape. The current study investigates the host’s immune response to TNBC cells and explores the presence of immunosuppressive factors, such as pAKT expression and infiltration with FoxP3 positive regulatory T-cells (Tregs), in human TNBC samples.

**Material and Methods**

NK-cell induced lysis of tumor cells was evaluated in human breast cancer cell lines MCF-7 (ER/PR pos.), HCC 1937 (triplenegative, BRCA 1 mutated) and HCC 1806 (triplenegative). Expression of pAKT and infiltration with Tregs was determined by immunohistochemistry and evaluated semiquantitatively (0 no expression - 3 strong expression). Control groups consisted of: Fibroadenoma (N=6), prophyliactic mastectomy (BRCA 1 mutated, N=3), ER/PR + breast cancer (N=13). They were compared with triplenegative breast cancers: N=9 BRCA wildtype and N=6 BRCA 1 mutated.

**Results**

At an effector target/target-ratio of 10:1 NK-cell induced lysis in HCC 1937 and HCC 1806 was 2.27 and 4.45 increased, respectively, as compared to MCF 7 cells. No infiltration with Tregs was detected in fibroadenoma and prophyliactic mastectomy samples. Infiltration with FoxP3-positive Tregs was 0.92 +/-0.75 in ER/PR + breast cancers and 2.66 +/-0.5 (p=0.05) in TNBC and 2.16 +/-0.98 (p=0.05) in TNBC with BRCA mutation, respectively. Expression of pAKT was 1.45 +/-1.29 in ER/PR + breast cancers and 1.77 +/-1.20 in TNBC and 2.66 +/-0.51 (p<0.05) TNBC/ with BRCA mutation.
Discussion
TNBC cells stimulated the NK-cell response to a stronger extent than did ER-positive MCF 7 cells mirrored by a more pronounced NK-cell-induced lysis. Thus, the observed stronger infiltration with FoxP3-positive Tregs in TNBC tumor samples could reflect a compensatory mechanism to suppress the host’s immune response. In other tumor entities, such as ovarian cancer infiltration with Tregs is associated with a worse overall survival (1). Thus, the significantly increased infiltration with Tregs, could suggest that the worse prognosis of TNBC is due to tumor immune escape and further investigation of immunomodulatory therapeutic strategies in TNBC could prove fruitful.

(1) Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival.


P1-01-07
ErbB-2 Peptide Vaccination Suppresses Spontaneous Tumorigenesis and Tumor Stem Cell Expansion in MMTV-PyVT Transgenic Mouse.

Park KH, Gil EY, Choi YJ, Kim ST, Cho KR, Seo JH, Lee ES, Kim JS, Disis ML. Korea University College of Medicine, Seoul, Korea; Korea University College of Medicine; University of Washington, Seattle, WA

Immunization targeting ErbB-2 could have considerable therapeutic potential by controlling growth and metastasis of highly aggressive tumor cells in the earlier preclinical and clinical studies. Just a few studies have examined preventive potential of ErbB-2 vaccines in preclinical studies. However, animal model systems used in the previous studies were tumor transplantation or neu-transgenic mouse, which were not relevant to human HER-2 positive breast tumorigenesis. In this study, active immunotherapy against tumor antigen ErbB-2/neu for primary prevention of breast cancer was tested using FVB/N-Tg (MMTV-PyVT) transgenic mice model. Mice were grouped to receive either ErbB-2 peptide vaccine, immune adjuvant only, tetanus toxoid, or PBS every 2 weeks for 3 months and monthly thereafter. The MMTV-PyVT transgenic mice in control groups (PBS, immune adjuvant only, or tetanus toxoid peptide) developed spontaneous mammary adenocarcinomas in 12 to 15 weeks, but vaccination against ErbB-2 strongly suppressed tumor formation by 30 weeks of observation. Further pathologic examination showed complete prevention of tumorigenesis was observed in ErbB-2 vaccinated mice, whereas the mice in control groups developed highly aggressive ErbB-2 overexpressing tumors similar to human breast cancer. The tumor protective effect of peptide vaccination was associated with induction of ErbB-2-specific humoral immune responses as well as T cell responses. Additionally, role of signal through ErbB-2 pathway and the relationship with stemness of cancer cells were determined by Aldefluor assay, mammosphere formation assay using Mouse mammary carcinoma (MMC) cells in vitro, and level of nestin expression determined by Western blot analysis. Further analysis of mammosphere formation capacity of MMC cells using immune sera showed that sera from ErbB2 vaccinated mice had a significant inhibitory effect on mammosphere formation in ErbB2 overexpressing MMC cells. These results suggest that ErbB-2 targeting by cancer vaccination might be useful adjuvant to standard therapy, helping to prevent relapse in patients with ErbB2-overexpressing tumors by suppressing stem/progenitor cell population.

P1-01-08
Expression of Interleukin-15 (IL-15) and the IL-15 Receptor in Human Breast Cancer.

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Introduction: Interleukin-15 (IL-15) is a cytokine that influences activation and proliferation of T-lymphocytes. IL-15 is produced in the body leukocytes such as phagocytes and in many ways has similar immunoregulatory functions to IL-2 including stimulation of NK cells and CD8(+) T cells. It has been suggested that IL-15 may increase the immunity to cancer cells and cancer cell’s response to therapeutic agents. IL-15 has also been shown to be able to inhibit tumour growth in vivo. However, the expression profile of IL-15 and IL-15 receptor (IL-15) in solid tumours including human breast cancer is not clear. The present study investigated the expression profile of both IL-15 and IL-15R in human breast cancer and deduced a clinical and pathological relevance with breast cancer.

Methods: Immunohistochemical methods were used to detect IL-15 and IL-15RA in mammary tissues. IL-15 and IL-15RA transcripts were analysed using real-time quantitative PCR method. Levels of IL-15 and IL-15RA were compared in normal and tumour tissues as well as against tumour staging, nodal status, disease progression and clinical outcome after a 10-year followup.

Results: Both IL-15 and IL-15RA were detectable in mammary tissues and were seen in both epithelial cells and infiltrating cells. Node positive tumours had low levels of IL-15 compared with node negative tumours (21.7±10 vs 103±46, respectively). Late stage tumour also had lower levels of IL-15 (95±43, 31±15, 3.7±2 and 1.3±1.5 for stage I, II, III and IV tumours respectively, p=0.036 and p=0.032, stage-II and Stage-III vs stage-I). Patients with metastatic disease (10.3±3) and patients died of breast cancer related conditions (54.7±25). The disease free survival time for patients with low levels IL-15 was 126 (114-138, 95%CI) months, compared with 139 (131-148) months for those with high levels of IL-15. Despite the reduced expression of IL-15 in aggressive tumours, expression of IL-15 receptor, IL-15RA, did not display a significant change and failed to showed a link with nodal status, tumour staging and clinical outcome.

Conclusions: Interleukin-15, an immunoregulatory cytokine, has an aberrant expression in human breast cancer. Low levels of IL-15, but not IL-15 receptor, is associated with the aggressiveness and disease progression of breast cancer. Together with reported effect of IL-15 on NK cells and other anti-tumour lymphocytes, IL-15 appears to be a useful therapeutic option.

P1-01-09
A Focused Immune Response Targeting the Homotypic Binding Domain of the Carcinoembryonic Antigen Blocks the Establishment of Tumor Foci In Vivo.

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Background: The carcinoembryonic antigen (CEA) is over-expressed in ~56% of breast cancer biopsies but is absent on normal breast tissues. Clinically, high preoperative serum concentrations of CEA in breast cancer patients correlate with metastasis, treatment failure and poor overall prognosis. More importantly, the homotypic cell adhesion functions of CEA have been associated with cancer progression and tumor metastasis. One approach to prevent the establishment of CEA-dependent metastatic foci in breast cancer patients would thus be to
interfere with the cell adhesion functions of this cell surface antigen. We hypothesize that vaccinating CEA-expressing transgenic (CEA-Tg) mice with a recombinant, altered-self form of the Ig V-like N domain of CEA, involved in homotypic interactions, should result in a focused immune response able to block the CEA-mediated adhesion of murine carcinoma cells expressing CEA (MC38.CEA) and the establishment of metastatic foci in this transgenic mouse model.

Material and Methods: A recombinant form of the CEA N domain was expressed in E. coli, purified and shown in cell-based assays to bind to the Aβ domain of human CEA and to block cell adhesion in CEA-expressing tumor cell lines. CEA-expressing transgenic mice were vaccinated i.p. with the recombinant CEA N domain protein and poly I:C. Resulting serum Ig [IgG1 and IgG2a] as well as cytokine expression levels [IFN-γ, IL-4 and IL10 responses] were measured by ELISA and ELISpot assays. The CEA-expressing MC38 murine tumor cells were implanted s.c. into CEA-Tg mice [hind leg; primary site model; tumor growth within 7 days] or administrated i.v. to generate lung tumor metastases within 60 days or injected i.p. to generate peritoneal tumor nodules within 35 days in unvaccinated animals.

Results: The recombinant folded, deglycosylated N domain of human CEA is perceived as an altered self-antigen by CEA-Tg mice when administered i.p. with poly I:C acting as an adjuvant. The vaccinated mice develop strong IgG1 and IgG2a responses able to kill CEA-expressing human breast cancer cell lines [MCF-7 and MDA-MB231] in vitro by both Antibody-Dependent Cellular Cytotoxicity (ADCC) and Complement-Dependent Cytotoxicity (CDC) mechanisms. The sera of such animals also inhibited cell-cell aggregation mediated by CEA expression. Vaccination of CEA-Tg mice following the establishment of CEA-expressing MC38 murine hind leg tumor mass resulted in a significant delay in tumor growth. More importantly, animals pre-vaccinated with the recombinant CEA N domain did not display lung or peritoneal tumor foci following the i.v. or i.p. injection of CEA-expressing murine tumor cells.

Discussion: A simple vaccination protocol using a recombinant form of the N domain of CEA as an immunogen is sufficient to engender an immune response that can block the development of CEA-expressing tumor foci in the lungs and peritoneal cavity of CEA-Tg mice and destroys human breast cancer cells expressing CEA. These results suggest that mounting a focused antibody response to the N domain of CEA may represent a simple therapeutic strategy to control the establishment of metastatic foci in breast cancer patients expression high levels of the antigen CEA.

P1-01-10
Immune Suppression of Regulatory T Cells and M2 Macrophage in Breast Cancer Patients.
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Background: Recent multi-disciplinary treatments of breast cancer (BC) could decrease the mortality rate, but successful immune therapy remains uncertain. To explore new strategy of immune therapy of BC, we are investigating about host-tumor immune response in BC patients. Materials and methods: Tumor tissue specimens and peripheral blood mononuclear cells (PBMC) were obtained from early and advanced BC patients. PBMC were also collected from healthy volunteers. Regulatory T (Treg) cells were examined by counting CD4+CD25highCD127low/-cells in PBMC with flow cytometry analysis. Immunohistochemical evaluation of tumor specimens was performed with monoclonal antibodies of HLA-ABC and DR, CD56, CD68, CD83, and CD163. The number of stained cells was analyzed using a semiquantitative ordinal scale ranging from 0 to 3 (0, +, ++, +++). Results: HLA-ABC and DR were stained negative in 44% and 82% of 50 BC cases. When host-tumor immune response were compared between 38 early BC cases and 12 advanced BC cases, numbers of CD68-positive cells significantly increased in peripheral tumor tissues of advanced BC cases than in those of early BC cases (92% versus 53%). CD163-positive tumor cells were also detected more frequently in advanced BC cases than in early BC cases (75% versus 16%). There were no significant differences of distribution of CD4, CD8, CD56, and CD83-positive cells in early and advanced BC cases. Treg cells in PBMC significantly increased in percentage of the population in 37 BC patients than in 21 healthy volunteers (4.2% versus 2.5% of CD4-positive cells at mean value). Interestingly, Treg cells decreased in percentage of the population in 36 postoperative BC patients. Conclusions: Our results suggest that Treg cells render BC patients under immune suppression and M2 macrophage plays an important role of tumor progression. Targeted therapy against M2 macrophage may be a promising strategy of breast cancer.

P1-01-11
CD4+CD25highCD127low/- Regulatory T Cells Have Immunosuppressive Function in Patients with Breast Cancer.
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CD4+CD25highFoxp3+ regulatory T cells play a central role in self-tolerance and suppress antitumor immune response. Recent studies have shown that low levels of the IL-7 receptor (CD127) are expressed on CD4+CD25highFoxp3+ regulatory T cells surfaces and the expression of CD127 is inversely correlated with the suppressive function of CD4+CD25high regulatory T cells. We evaluated the frequency of CD4+CD25highCD127low- T cells in peripheral blood lymphocytes of 72 breast cancer patients and 21 healthy volunteers. The expression of Foxp3 mRNA was inversely correlated with the CD127 expression and correlated with CD25 expression. The frequency of CD4+CD25highCD127low- T cells in breast cancer patents was significantly higher than that of healthy volunteers (p=0.0045). CD4+CD25highCD127low- T cells were increased in hormone receptor negative patients and HER2 positive patients, but no statistical significant was observed in stage progression. In addition, the frequency of CD4+CD25highCD127low- T cells decreased after curative resection in breast cancer patients (p=0.005). CD4+CD25highCD127low- T cells also suppress proliferation of autologous CD4+CD25+ helper T cells and CD8+ cytotoxic T cells in CFSE-based cell proliferation assay. These findings suggest that CD4+CD25highCD127low- T cells a useful marker for regulatory T cells in breast cancer. Circutious peripheral regulatory T cells may participate in immune tolerance to breast carcinoma.

P1-01-12
Mesoporous Silicon Particles for the Presentation of Tumor Antigens and Adjuvant for Anti-Cancer Immunity.
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Custom-made vaccines based on personalized tumor antigens are realistic options for secondary therapy, with the patient’s excised tumor providing antigens. Overlay in the personalized repertoire of tumor antigens among patients also provides insight into targets for preventative cancer vaccines. Nanotechnology provides carriers for
shielded delivery of antigens and presentation of immunostimulatory molecules. Rapid uptake of particles by phagocytic immune cells and migration to lymphatic tissue for antigen presentation provides opportunities to elicit tumor specific immune responses. Dendritic cells (DC) are the master antigen presenting cells (APC) for efficient processing and presentation of antigens. We have tested the ability of mesoporous silicon particles (pSi) to function as substrates for DC and to activate cells via engagement of surface toll-like receptors (TLR). pSi particles, surface labeled with lipopolysaccharide (LPS) or monophosphoryl lipid (MPL), were presented to bone marrow-derived DC. This resulted in rapid uptake of particles, with an enormous capacity for number of particles internalized per cell. Confocal microscopy studies supported higher uptake of LPS and MPL conjugated as compared to unlabeled pSi. pSi particle uptake into DC was also supported by electron microscopy imaging. TLR ligands induced morphological changes in GM-CSF stimulated cells consistent with maturation towards a DC phenotype. As expected, LPS conjugation to particles resulted in significant toxicity. MPL conjugated pSi showed little or no toxicity to DC and showed improved particle uptake into DC with morphological changes consistent with maturation towards a mature DC phenotype. The impact of cytokine cocktail on DC maturation and its impact on particle uptake were also examined. Flow cytometry analysis supported greater uptake of pSi by DC stimulated with GM-CSF and IL-4 compared to GM-CSF alone, with reduced uptake in the presence of TNF-alpha. Consistent with findings reported by others, addition of TNF-alpha to the cell media also resulted in higher levels of expression of costimulatory molecules by DC, compared to cells stimulated with GM-CSF alone. Thus immature DC are better able to internalize particle-based vaccines, with cytokine or TLR-driven maturation enhancing expression of costimulatory molecules for more effective antigen presentation.

P1-01-13
Prognostic Impact of CD8 in Node-Negative Breast Cancer.
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Background: Infiltration of cytotoxic T lymphocytes (CTL) is a common feature in breast cancer. We examined the prognostic impact of CD8 positive CTL in formalin-fixed-paraffin-embedded (FFPE) tissue using immunohistochemistry (IHC) in node-negative breast cancer and validated our findings in previously published cohorts using RNA expression.

Methods: CD8 was evaluated in FFPE tissue of 339 medically untreated node-negative breast cancer patients utilizing IHC. Results were validated utilizing microarray based gene-expression data of four cohorts of medically untreated node-negative breast cancer patients (n=824). Impact of CD8 on metastasis-free survival (MFS) was analyzed with univariate and multivariate Cox regression. Meta-analysis of previously published cohorts was performed using a random effects model. Prognostic significance was examined in the whole cohort and in different molecular subtypes (ER+/HER2-, ER-/HER2-, HER2). Correlation between RNA expression and IHC was analyzed according to Spearman.

Results: Immunohistochemical detection of CD8+ CTL was associated with MFS in univariate (hazard ratio [HR] 0.71, 95% CI 0.51-0.99, P=0.0201) and HER2+ (HR 0.60, 95% CI 0.38-0.97, P=0.037) as well as in multivariate analysis (HR 0.77, 95% CI 0.52-1.14, P=0.1965). Conclusion: CD8 positive CTL have independent prognostic significance in node-negative breast cancer.

P1-01-14
Gene Expression of Immune Mediators within Nipple Aspirate Fluid and Ductal Lavage from Normal and Cancerous Breasts.
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The majority of breast cancers are thought to initiate in the lining of the 6 to 8 ducts that exit the nipple. Evidence suggests that the presence of nipple aspirate fluid (NAF), and in particular NAF containing atypical epithelial cells, in the ducts is predictive of future breast cancer risk. Although NAF fluid and cells may be representative of a preneoplastic state, use of NAF has not proven to represent a robust indicator for future malignancy. Chronic infiltration of tissues by immune cells predisposes them to future malignancy. Since macrophages can represent up to half of the total cells recovered from NAF, and their presence within malignant breast tumors has predictive value in terms of survival, we hypothesized that changes in the inflammatory microenvironment of breast ducts may foster future malignancy and/or provide an early indicator of future neoplasia. To test this hypothesis, we conducted an exploratory study to investigate gene expression in NAF and ductal lavage fluid. We first used real-time PCR to quantitatively measure mRNA expression of select genes within the cellular milieu of NAF to determine if that microenvironment correlated with a tumor-promoting state. Expression of multiple genes was observed in 15 samples collected from healthy volunteers, with robust expression of myeloid-associated genes including CD14, CD68, CCL3, CSF1 and IL1B observed in the majority of samples. To determine whether similar inflammatory microenvironments were found within each duct, and whether they were comparable to those in NAF-containing ducts, ductal lavage (DL) samples were obtained from 4 different ducts at the same time as NAF collection. Out of 7 volunteers, 4 provided samples allowing comparison between NAF and DL, and between multiple ducts. Analysis of gene expression from these healthy volunteers indicated that gene expression differed between NAF samples and each DL sample. More importantly, unique gene expression was found for each duct sampled, indicating that the immune microenvironment within each duct is distinct from other ducts within the same breast. In order to identify a potential inflammatory gene signature, we then obtained NAF samples from patients with breast cancer that had not received neoadjuvant chemotherapy prior to surgery, from both the cancerous and contralateral breasts. With 4 patients analyzed, no consistent expression difference has thus far been observed, indicating either that the relevant genes have not been identified, or that analysis of inflammatory genes within NAF may not be a predictive method with which to evaluate risk of future malignancy in women that produce NAF. A larger study is warranted to further investigate the significance of inflammatory gene expression in breast ductal fluid.
P1-01-15
Do Serum Cytokines Predict Breast Cancer Behavior?
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Introduction: The role of inflammation in breast cancer development and progression is poorly understood. The potential for serum cytokines to predict breast cancer behavior is an intriguing hypothesis, and data to date, has been conflicting. We sought to determine if serum inflammatory cytokines could predict clinicopathologic factors in breast cancer patients.

Methods: Fifty-seven women with newly diagnosed breast cancer (stage 0-4) were accrued to this IRB-approved study. Serum cytokine levels were obtained in 50 of these women; this was the cohort of interest. All patients underwent surgical therapy, and log serum cytokine levels were correlated to final pathologic results.

Results: The median patient age was 51 (range: 21-79) with a median tumor size was 19.5 mm (range: 2.3-95.0 mm). A variety of cytokines were evaluated. None were found to correlate with tumor size, lymph node status, presence of metastases, tumor grade, or lymphovascular invasion (all p's > .05). While most of the cytokines evaluated did not correlate with hormone receptor status, a significant correlation was seen between serum IFN-gamma and estrogen receptor (p = .011) and progesterone receptor (p = .013) status. No correlation was seen with her-2-neu status (p > .05).

Conclusions: Serum cytokine levels do not seem to correlate with tumor size, lymph node status or grade. However, the strong correlation between IFN-gamma serum levels and hormone receptor status is hypothesis-generating and requires further study.

P1-01-16
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Background:
Noninvasive biomarkers for the early detection of breast cancer are crucial due to the fact that the relapse risk of breast cancer is rising with the time point of its detection. Currently, none of the reported molecular biomarkers is established for the clinical use as a diagnostic tool. Previous proteomics-based studies showed the immunogenicity of breast carcinoma and the following B-cell mediated immune response. As a result, several autoantibodies against tumor proteins were detected in the sera of breast cancer patients. However, these putative biomarkers are still lacking of clinically reliable specificity and sensitivity, even of better discrimination of cancer patients when combining different biomarkers. The search for a new antibody biomarker signature remains very important as a potential cancer detection tool. For further investigations we analyzed the antibody pattern in serum samples of diseased patients and healthy controls after incubation with whole protein extract from a native carcinoma and identified the putative tumor-specific immunoreactive antigens.

Materials and methods:
For our de novo profiling of tumor antigens we used a protein extract from a primary invasive ductal carcinoma. Sera from 20 women, of which 19 were diagnosed with breast carcinoma and one with DCIS (CA), and 20 sera from age-matched healthy donors (CTRL) were obtained and pooled separately. For optimal separation of tumor antigens a two-dimensional sodium dodecylsulfate gel electrophoresis (2D SDS-PAGE) was applied. Following immunoblots with each serum pool were performed and the immunospecific reactions visualized with a horseradish peroxidase-conjugated anti-IgG antibody. The relevant tumor antigens were identified via Matrix-Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI TOF-TOF MS).

Discussion:
We identified over 10 tumor antigens which reacted with the corresponding autoantibodies in CA or CTRL approach, showing again the complexity of immune response. Besides of already described breast carcinoma related antigens like alpha enolase, which showed immune reaction also with the healthy serum pool, we identified several potential antigens of interest like peroxiredoxin 6 which showed a strong immune response only after the incubation with cancer sera.

P1-02-01
c-Jun N-Terminal Kinase 1 (JNK1) Inhibits Tumor Growth and Metastasis by Downregulating Epithelial to Mesenchymal Transition (EMT) and Stem Cell-Related Genes.
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Murine and human mammary cancers often show dysregulation of important signaling pathways including the canonical Wingless-iNtegrated (Wnt) and avian ERythroBlastosis oncogene B (ErbB) pathways. Transgenic expression of Wnt ligands causes transformation of normal mammary cells in mice, and Wnt10b is frequently upregulated in human breast cancers. Overexpression of ErbB ligands and amplification of receptors have also been implicated in human breast cancer. JNK1 is a tumor suppressor in the skin and intestinal epithelium, and JNKs are known to integrate ErbB and Wnt pathways as well as others to control cell growth and differentiation. In a murine mammary cancer model where 4T1.2 cells were injected into the mammary gland, reducing JNK1 levels by expressing shRNA (shJNK1) resulted in increased tumor growth and lung metastasis compared to mice injected with control vector-(pSM2) expressing cells. A microarray analysis comparing gene expression between shJNK1 and control tumors revealed 2 and 2.5-fold increases in the ErbB pathway genes Nrg3 and ETV5, 2-fold increases in the Wnt genes Bcl-9 and Wnt10a, and a 1.6-fold increase in the EMT gene Twist1. RT-PCR analysis of in vitro grown 4T1.2 cell lines transfected with shJNK1 confirmed increased expression of Bcl-9 and ETV5, as well as the ETV5 target Cox-2. ErbB2 protein was also overexpressed. The shJNK1 cells showed upregulation of pERK in response to Herregulin1 (an ErbB2/3 ligand) and Fibroblast Growth Factor (FGF) 1, which further amplifies canonical Wnt signaling. In a p53-/- tumor model, ETV5, Bcl-9, and Cox-2 were still upregulated in jnk1-/- compared to wildtype tumor cells, indicating this effect is p53-independent. In the normal mammary gland, a 4-fold increase in ETV5 and a 5-fold increase in Twist1 were found in jnk1-/- mice.
compared to wildtype, further indicating that this effect is dependent on JNK1 alone. The ErbB and Wnt pathways are known to upregulate EMT and stem-cell related genes, however, the involvement of JNK1 in these effects is a novel hypothesis. Thus far, our data suggest that JNK1 deficiency targets these oncogenic pathways to contribute to a more aggressive tumor phenotype due to heightened EMT and “stem-cellness”. Further studies using cell sorting and differentiation assays will determine whether normal jnk1−/- glands contain a higher fraction of stem cells than wildtype glands. Inhibition of EMT genes and/or ETV5 in the p53−/−,jnk1−/- cancer cells will determine whether the tumor growth or metastasis phenotypes are dependent on these genes. EMT and stem-cell genes are frequently expressed in human breast cancer subtypes that exhibit low survival rates, and EMT is known to be linked to increased metastasis. Some of these sub-types, such as claudin-low tumors, currently have no molecularly-targeted treatments, therefore it is important to determine what proteins critically contribute to these phenotypes so that efficient and effective treatments can be developed.

P1-02-02
Zoledronic Acid Reverses the Epithelial-Mesenchymal Transition While Inhibiting the Tumor Initiating Cell Population of Highly Tumorigenic Breast Cancer Cell Lines.
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Breast cancer remains the second leading cause of cancer related death amongst women in the United States. This is largely due to metastasis of cancer cells from the primary tumor to other parts of the body, and to the putative cancer stem cell population. Both are driven by the epithelial-mesenchymal transition (EMT), a cellular process whereby cancer cells of epithelial origin lose their epithelial characteristics and acquire a mesenchymal phenotype. Cells which undergo EMT tend to be motile and invasive, and therefore can metastasize to other parts of the body. EMT has also been implicated in the generation of cells expressing the cancer stem cell phenotype. As metastatic disease and the cancer stem cell are difficult to eliminate, more effective therapy is required. Zoledronic acid, originally indicated for use in the treatment of osteoporosis, has been reported to inhibit the growth of breast cancer cells. The mechanism of this effect however has yet to be determined. In preliminary data studies, treatment with zoledronic acid was found to reduce activation of nuclear factor kappa-b, an established regulator of EMT. These findings suggest that treatment with zoledronic acid may reverse EMT in breast cancer cells, driving them to express a more epithelial phenotype. To test this hypothesis, the highly metastatic, triple negative breast cancer cell lines MDA-MB-231 and Hs578t, which largely express mesenchymal proteins, were used to measure cell viability and changes in protein and mRNA expression following treatment with zoledronic acid. Dose response analysis for inhibition of cell viability showed an IC50 of approximately 2µM in the Hs578t and 6 µM in the MDA-MB-231 cell line. Zoledronic acid treated cells displayed a decreased mesenchymal phenotype, as evidenced by reduced expression of mesenchymal markers N-cadherin (75% reduction, p<0.0076; 65% reduction, p=0.005) and TWIST (67% reduction, p=0.08; 64% reduction, p=0.009) in MDA-MB-231 and Hs578t cells, respectively. This was accompanied by a subsequent increase in epithelial phenotype as evidenced by increased expression of epithelial marker E-cadherin (223% increase, p<0.006) in MDA-MB-231 cells. To further elucidate the effects on the mesenchymal and epithelial phenotypes of these cells, surface expression of CD24 and CD44 was measured by flow cytometry. While vehicle treated samples of both cell lines stained positive for CD44 and negative for CD24, zoledronic acid treatment decreased CD44 expression. As both increased the ratio of surface expression of CD44hi/CD24lo and mammosphere formation are characteristics of the breast cancer stem cell, effects of zoledronic acid on this subpopulation in Hs578t cells were determined. Cells pretreated with zoledronic acid were seeded under mammosphere conditions and allowed to propagate for 7 days. Zoledronic acid treated cells formed significantly fewer mammospheres (86% reduction, p<0.041), while the ones that formed were smaller in size. These findings suggest that zoledronic acid is able to reverse the epithelial mesenchymal transition, which may reduce the tumor initiating capacity of highly metastatic cells.

P1-02-03
The Reciprocal Roles of E-Cadherin and ZEB1 Demonstrate the Mesenchymal-Epithelial Transition as a Primary Characteristic of Inflammatory Breast Cancer.
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Background: Inflammatory breast cancer (IBC) is a rare but very aggressive form of breast cancer. IBC is characterized by nests of tightly aggregated cells, defined as tumor emboli, that exhibit characteristics of cancer stem cells (CSCs). IBC tumor emboli express E-cadherin which is required to maintain their integrity and our recent evidence demonstrates that expression of E-cadherin by tumor emboli is associated with lack of ZEB1 expression, a transcriptional repressor of E-cadherin. This is at odds with the current hypothesis that metastatic progression is associated with the process of epithelial mesenchymal transition (EMT), with loss of E-cadherin and gain of transcription factors including ZEB1, acquisition of CSC characteristics and enhanced invasive capabilities.

Materials and Methods: shRNA knockdown and over-expression methods, real time PCR arrays, western blotting, and in vivo assays to evaluate proliferation, invasion, growth in soft agar and clonogenicity and in vivo animal studies were used.

Results: Expression of E-cadherin was reduced by shRNA and ZEB1 was expressed in SUM149 IBC tumor cells. Numerous EMT-related genes were upregulated with loss of E-cadherin and gain of ZEB1, including N-cadherin and vimentin. However, there were marginal differences in the in vitro parameters of proliferation, Matrigel invasion and anchorage independent growth in soft agar between SUM149-shEcad or SUM149-ZEB1 clones and their respective vector control cells. The loss of E-cadherin and gain of ZEB1 altered the morphology of SUM149 cells when cultured under low adherence conditions permissive for the enrichment of CSC, exhibiting a reversion in grape-like morphology to more well defined spheres, which was accompanied by increased clonogenicity in both SUM149-shEcad and SUM149-ZEB1 cells. The loss of E-cadherin and the gain of ZEB1 significantly inhibited tumor growth of cells injected in the mammary fat pad of NOD.Cg-PkdcreloxP129+Il2rgtm1Wjl/SzJ mice. Tumor volume at 56 days for E-cadherin vector control cells was 771.9 mm3 +/- 185.6 compared to shEcadherin tumors, which was 13.6 mm3 +/- 7.2. Tumor volume of ZEB1 expressing tumors, which was 346.1 mm3 +/- 96 compared to volume of ZEB1 expressing tumors, which was 21.5 mm3 +/- 7.2.

Conclusions: E-cadherin with lack of ZEB1 expression in IBC is consistent with a mesenchymal-epithelial transition (MET), consistent with the retention of the epithelial phenotype while maintaining a program of rapid metastasis and colonization of lymph nodes and distant organ sites. Furthermore, we demonstrate that the E-cadherin-ZEB1 axis is critical for the in
vivo growth of IBC tumor cells. Although SUM149 cells are fully capable of undergoing an EMT process, which is under negative regulation by E-cadherin, the process of EMT does not drive in vivo tumor growth in IBC.

**P1-02-04**

**Estrogen Receptor beta Inhibits Breast Cancer EMT by Regulating the Expression of miR-200.**

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Estrogen receptor beta (ERβ) mediates the effects of estrogens in a variety of human tissues and regulates cellular processes involved in initiation and progression of breast cancer such as cell proliferation and migration. Clinical studies produced contradictory data regarding the role for ERβ in prognosis of metastatic breast cancer and the molecular mechanism through which ERβ influences cell migration and invasion has not been fully elucidated. Here we show that induction of ERβ expression inhibits epithelial to mesenchymal transition (EMT) in metastatic breast cancer cells. This correlates with an ERβ-mediated induction in the expression of the epithelial marker E-cadherin and downregulation of its transcriptional repressors ZEB1 and SIP1. ERβ alters the expression of ZEB1 and SIP1 by inducing the expression of the miR-200a, miR200b and miR-429. Downregulation of these miRNAs in ERβ-expressing cells resulted in decreased cell-cell contact and decline of E-cadherin levels. In addition, ERβ was found to inhibit the invasiveness of metastatic breast cancer cells in a zebrafish xenotransplantation model. We are now examining breast cancer specimens derived from ductal carcinomas and metaplastic breast cancers to see whether ERβ levels decline in the mesenchymal regions and ERβ expression is correlated with epithelial markers. These data propose a crucial role for ERβ in the regulation of EMT and in prognosis of invasive and metastatic breast cancer.

**P1-02-05**

**Invasive Lobular Carcinoma – A Luminal Breast Cancer Histotype Enriched for Epithelial-to-Mesenchymal Transition Features.**

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Background: Invasive lobular carcinoma (ILC) represent the second most common breast cancer (BC) histotype, but little information remains a statistically significant prognostic factor for ILC. ILC tumors were enriched with an EMT phenotype, which is not observed among IDC luminals. Of interest IGF signaling, which is known to be associated with poor prognosis in ER-positive BC, added prognostic value in this population which may open new therapeutic avenues for ILC.

Conclusions: ILC is mainly composed of luminal tumors and a minority of HER2-positive tumors. Similarly to IDC, proliferation remains a statistically significant prognostic factor for ILC. ILC tumors were enriched with an EMT phenotype, which is not observed among IDC luminals. Of interest IGF signaling, which is known to be associated with poor prognosis in ER-positive BC, added prognostic value in this population which may open new therapeutic avenues for ILC.

**P1-02-06**

**Silencing of IGF-1R Has Paradoxic Effects in Triple Negative Breast Cancer Phenotypes.**

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Background: Triple negative (TN) breast cancers are a heterogeneous group of breast cancers with a poor prognosis in part due to a lack of effective targeted agents. Insulin-like growth factor-1 receptor (IGF-1R) has been shown to play a role in breast cancer cell proliferation, adhesion, invasion, and migration and is overexpressed in more than a third of TN breast tumors. We hypothesized that IGF-1R could be a therapeutic target for a subset of TN breast cancers. Methods: We evaluated the effects of IGF-1R silencing on the metastatic properties of TN breast cancer cells by knock down in two morphologically distinct TN breast cancer cell lines using shRNA lentiviral techniques. Reverse-transcription polymerase chain reaction (RT-PCR) and immunoblotting were used to detect mRNA and protein expression levels, respectively, of IGF-1R signaling molecules. Anchorage-dependent growth and Matrigel chamber assays were performed to assess the effects of IGF-1R silencing on colony formation and invasion of TN breast cancer cells, respectively; wound-healing and spheroid migration were also performed to assess the effects of IGF-1R inhibition on TN breast cancer cell motility. Results: Stably transfected mesenchymal MDA-MB-231 TN cells showed effective downregulation of IGF-1R protein expression, which resulted in mesenchymal-to-epithelial transition (MET), confirmed by
upregulation of the epithelial marker E-cadherin and downregulation of the mesenchymal marker vimentin. Importantly, this MET resulted in reduced colony formation (p<0.0042) and cell motility and dramatically reduced invasion (p<0.0001). Conversely, silencing of IGF-1R in epithelial MDA-MB-468 TN cells induced epithelial-to-mesenchymal transition (EMT), confirmed by downregulation of E-cadherin and upregulation of vimentin expression, with resultant increased colony formation (p<0.006), cell motility, and invasion (p<0.0001). Conclusion: Collectively, these results demonstrate a paradoxical effect of targeting IGF-1R in TN breast cancers of mesenchymal and epithelial origin. Targeting IGF-1R in TN breast cancers with a mesenchymal phenotype decreases invasion and metastatic potential. In contrast, targeting IGF-1R in TN breast cancers with an epithelial phenotype could have potentially detrimental effects. Our data suggest that IGF-1R inhibition should be explored as a therapeutic modality in TN breast cancers with a mesenchymal phenotype.

P1-02-07 Epithelial-Mesenchymal Transition Correlated with Serum Cytokine Profiling in Breast Cancer Patients.
Giordano A, Cohen EN, Anfossi S, Gao H, Lee B-N, Mego M, Sanda T, Valero V, Alvarez RH, Cristofanilli M, De Placido S, Horta obese GN, Woodward W, Ueno NT, Reuben JM. The University of Texas MD Anderson Cancer Center, Houston, TX; University Federico II of Naples, Naples, Italy; Fox Chase Cancer Center, Philadelphia, PA; National Cancer Institute, Bratislava, Slovakia (Slovak Republic)

Background: Inflammation contributes to the increased invasiveness and poor prognosis in breast cancer (BC) patients. Specifically, the expression of the proinflammatory cytokines interleukin-6 (IL-6), tumor necrosis factor alpha (TNFα) and interleukin-1 (IL-1) have all been linked to increased invasiveness and poor prognosis. Interestingly, the increased invasiveness was associated with an increase in the acquisition of markers of epithelial-mesenchymal transition (EMT). Therefore, we determined whether the levels of circulating proinflammatory cytokines (IL-1, IL-6, TNFα) and anti-inflammatory cytokines (IL10) were correlated with the induction of EMT transcription factors (TFs), Snail1, Zeb1, Twist1, in breast cancer patients.

Materials and Methods: From two laboratory-based ongoing studies at the MD Anderson Cancer Center, 41 BC patients were assessed for EMT-TFs in circulating CD45-ve cells (EMT-CTCs) and for serum proinflammatory cytokines before starting any treatment. 32 of 41 patients assessed for EMT had metastatic BC. EMT-CTCs were detected by qRT-PCR for the EMT-TFs Snail1, Zeb1 and Twist1 (Mego 2011; PMID 21387303) and serum cytokines were measured by Luminex bead array assay (MILLIPLEX™ MAP Human Cytokine/Chemokine Panel). Cytokine serum concentrations were compared with the median cytokine levels of healthy donors (HD). We examined the association of serum cytokines above the median HD levels and the presence of EMT-CTCs by non-parametric Mann-Whitney test with a statistical significance for p<.05.

Results: Of the 41 patients assessed for both serum cytokines and EMT-CTCs, 14 (34%) were positive for at least one EMT-TF, including 3 of 9 (33%) patients with no-metastatic BC and 11 of 32 (34%) patients with metastatic BC. We found that serum levels of IL1a, IL2, TGFα, and TNFβ in patients that were above the median levels of HD sera were higher in patients with EMT-CTCs in the blood (higher IL1a concentration in patients with over expression of Snail1, Zeb1, and Twist1; IL2 with Zeb1; TGFα with Snail1; TNFβ with Zeb1, and Twist1). Further, the higher ratio of proinflammatory/anti-inflammatory cytokines, was associated with the presence of at least one EMT-TF, e.g., IL8/IL10 (p=.005) and TNFα/IL10 (p=.037).

Discussion: Patients with proinflammatory cytokine (IL1a, IL2, TGFα, and TNFβ) levels above the median levels of HD or who had a predominantly proinflammatory cytokine profile were more likely to have at least one EMT-TF in their blood. These data are consistent with the hypothesis that proinflammatory cytokines promote EMT, which may be involved in tumor aggressiveness and disease progression.

P1-02-08 Modulation of EMT by Targeting E-Cadherin Restores Radiation Sensitivity in Human Breast Cancer Cells.
Munshi A, Yuan Y, Liu J, Meyn RE. University of Oklahoma Health Sciences Center, Oklahoma City, OK; Baylor College of Medicine, Houston, TX; The University of Texas M.D. Anderson Cancer Center, Houston, TX

E-cadherin is the major adhesion protein associated with epithelial cells and loss of its expression is diagnostic of the epithelial to mesenchymal transition (EMT). Findings from our laboratory, suggest that EMT, detected as the loss of E-cadherin expression, may regulate tumor cell radioresistance, i.e. cells that have undergone EMT are relatively radioresistant compared to the lines that have retained the epithelial phenotype, which typically are relatively radiosensitive. To definitively test this hypothesis we compared expression levels of E-cadherin and other EMT related markers in ER-a negative (MDA-MB-231 and Hs578t) and ER-a positive (MCF-7) human breast cancer cells. We observed that many ER-a negative lines, were also E-cadherin negative, and the ER-a positive lines were positive for E-cadherin expression. Clonogenic cell survival assays showed that the cell lines expressing estrogen receptor (MCF-7) and had high expression of E-cadherin, were also more sensitive to increasing doses of radiation. Treatment of MCF-7 cells with 100nM siRNA to E-cadherin for 72 hrs made the cells more resistant to radiation with the surviving fraction changing from 42.3% in control cells to 56.4% in siRNA treated cells. In contrast, ER negative cells (MDA-MB-231 and Hs578t) had no detectable expression of E-cadherin and were more radioresistant. We transfected MDA-MB-231 and Hs578t cells with a CDH1-expression vector and isolated stable clones, which were then tested for radiosensitivity. Cells transfected with the backbone vector (pCMV) served as controls. Restoring E-cadherin expression radiosensitized both, MDA-MB-231 and Hs578t cells, compared to the control vector cell line, suggesting that restoration of E-cadherin expression in mesenchymal-like cells produces a radiosensitizing effect. These observations suggest that ER-a signaling might regulate E-cadherin and implied that this regulation might influence EMT and radioresistance. Overall, our results suggest that the EMT status of breast cancer cells governs their response to radiation. A detailed investigation of the mechanism is ongoing.

P1-02-09 Epigenetics-Regulated microRNAs Related with Epithelial-Mesenchymal Transition of Breast Cancer Cells.
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Background: Epithelial-mesenchymal transition (EMT) is a program in which biological cells change morphologically and functionally from an epithelial phenotype to a mesenchymal phenotype. The EMT is involved in the process of cancer metastasis. On the other hand, accumulated evidence showed epigenetics and microRNA plays
important roles in breast cancer. However, to date, biological networks between epigenetics and microRNAs regarding EMT remains largely unclear. Thus, the aim of this study is to identify epigenetics-regulated microRNAs related with EMT of breast cancer.

Materials / Methods and Results: MicroRNA expression profiles of 11 breast cancer cell lines (8 epithelial-phenotype (E-type) cells and 3 mesenchymal-phenotype (M-type) cells) were obtained by microarray method. Unsupervised clustering analysis showed that E- and M-type breast cancer cells had different microRNA expression profiles. On the other hand, we obtained a genomewide methylation status of these breast cancer cell lines by a MeDIP-seq method. An integrated in silico analysis identified microRNAs which microRNA and DNA methylation were inversely correlated. All of miR-200b/a/429 cluster members were listed in top5 differentially expressed miRs between E- and M-type cells, and also in top 5 epigenetics-regulated microRNAs. In the further study, we focused upon the miR-200b/a/429 cluster. A COBRA assay revealed that promoter regions of miR-200b/a/429 cluster in M-type breast cancer cells were more frequently methylated than that in E-type cells (65.1% vs 6.8%, p<0.0001, respectively). The methylation levels were inversely associated with miR-200b/a/429 cluster expression (Taqman assay, p<0.01). In addition, demethylating treatment using 5-aza-dC unmasked miR-200b/a/429 expression in M-typed breast cancer cell lines. Taken together, the finding indicated that the expression of miR-200b/a/429 cluster is epigenetically regulated. Next, we investigated an effect of the miR-200b/a/429 cluster upon cell motility as a function of the EMT. A transfection of exogenous miR-200b/429 inhibited 24% of cell migration ability (Transwell assay). Utilizing microRNA target prediction algorithm, we identified fibronectin as a target gene of miR-200b/429. Utilizing several prediction algorithms for microRNA target genes, we identified fibronectin as a target gene of miR-200b/429. A luciferase-based reporter assay demonstrated that miR-200b/429 were directly associated with fibronectin-3'UTR and repressed 21% (p<0.0001) of the reporter gene expression post-transcriptionally. Conclusion: The promoter hypermethylation of miR-200b cluster is associated with epithelial-mesenchymal transition, and stimulates cell motility by upregulating fibronectin expression in mesenchymal-phenotype breast cancer cells.

P1-02-10
Vimentin Expression; as a Prognostic Factor and a Possible Molecular Target of Triple Negative Breast Cancer.

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BACKGROUND: Triple negative breast cancer (TNBC), characterized by absence of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2), is typically associated with aggressive tumor phenotype and poor prognosis. TNBC is also considered highly heterogeneous disease. Since TNBC lacks efficient therapeutic target, it is generally treated with non-specific cytotoxic agents. A better understanding of molecular and histopathological features of TNBC is of great importance, in order to develop a new therapeutic strategy and to improve the prognosis of TNBC. TNBC has many similarities with basal-like breast cancer (BLBC), and is also associated with stemness and BRCA-ness. In addition, recent studies suggest links between TNBC and epithelial-mesenchymal transition (EMT). To identify prognostic biomarkers and novel therapeutic targets, we investigated the expression of the factors associated with EMT in TNBC.

MATERIALS and METHODS: Sporadic invasive breast cancer specimens were obtained from 659 Japanese patients who underwent surgery in our department between 1994 and 2010, and 90(14%) cases were diagnosed as TNBC. The E-cadherin and vimentin mRNA expression was evaluated by quantitative RT-PCR. The E-cadherin, vimentin, CK5/6 and epidermal growth factor receptor (EGFR) protein expression was assessed by immunohistochemistry. In this study, we defined TNBC with positive expression of CK5/6 and/or EGFR as BLBC, and TNBC with low expression of E-cadherin and positive expression of vimentin as EMT-type.

RESULTS: Compared with non-TNBC cases, E-cadherin mRNA expression was significantly lower in TNBC cases (p=0.0012). Immunohistochemically, E-cadherin expression was significantly lower (p=0.0001) and vimentin expression was significantly higher (p=0.0049) in TNBC cases. Vimentin expression was associated with younger age (<50 years old, p=0.021), high nuclear grade (p=0.017) and high Ki67 expression (p<0.001) in TNBC. Among the patients with TNBC, vimentin expression was significantly associated with poor prognosis, in terms of disease free survival (p=0.0059) and overall survival (p=0.013). Multivariate analysis showed that vimentin expression was an independent prognostic factor for both disease free survival (p=0.017) and overall survival (p=0.012). Among TNBC cases, 52(63%) cases were BLBCs and 15(18%) cases were EMT-type. Among BLBC patients, vimentin expression was also associated with significantly shorter disease free survival (p=0.0058) and overall survival (p=0.0057). The patients with both BLBC and EMT-type features showed especially poor prognosis (p=0.05).

CONCLUSION: These findings suggest that elevated expression of vimentin attributes to the aggressive phenotype in TNBC patients. Vimentin expression might be useful as a molecular marker for prognosis of TNBC, and vimentin may represent a novel therapeutic target of TNBC.

P1-02-11
The BCL2 Antagonist of Death, BAD Is Down-Regulated in Breast Cancer and Inhibits Cancer Cell Invasion.

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Background

Recent clinical evidence suggest that expression of BCL2 and its antagonist BAD are good prognosticators for survival in breast cancer patients. BAD protein was previously shown by us to inhibit cyclin D1 expression and c-Jun activation, both may enhance invasiveness of tumor cells(Fernando et al JBC 07). However, the role of BAD and other BCL2 family proteins in invasion/metastasis of breast cancer is poorly characterized.

Methods

By immunohistochemistry nuclear and cytoplasmic staining of several proteins including BAD in normal epithelial was compared to that of cancer cells in human specimens . Western blotting and ELISA methods were used to compare BAD overexpressing MCF-7 breast cancer cells with control cells for the expression of variety of signaling molecules, proteins that take part in metastasis and invasion and ability of cells to invade was measured. PCR was used to measure mRNA levels and reporter constructs utilized for transcriptional factor activity studies.

Results

Grade II breast cancer specimens express less total and phosphorylated forms of BAD in nuclei and cytoplasm compared to normals. BAD expression decreased Sp1, β-catenin and STAT proteins, which may increase cyclin D1 in vitro. BAD inhibited activation of the CRE and
AP1 elements, and phosphorylation of BAD on S112 and S136 is required for this activity. BAD inhibited the Ras/MEK/ERK pathway and JNK, which may underlie inactivation of c-Jun. BAD, like BCL2, may decrease metastasis, therefore, ability of BAD to modulate the expression of metastasis-related proteins were measured and MMP10, VEGF, SNAIL, CXCR4, E-cadherin, and TIMP2 were regulated by BAD with a concomitant reduction in cell invasion.

Conclusion

Higher expression of BAD in breast cancer and a role in metastasis and proliferation suggests that BAD is a valuable diagnostic marker in breast cancer and a multi-functional protein. Further given the overwhelming clinical evidence that BCL2 prolongs survival, a reevaluation of the role of BCL2 family proteins in metastasis is urgently required.

P1-03-01

ECM Stiffness and Breast Tumor Histology and Treatment Phenotype.
Acerbi I, Au A, Chen Y-Y, Park C, Hwang S, Weaver V. Center for Bioengineering and Tissue Regeneration, University of California, San Francisco, San Francisco, CA; Carol Buck Breast Cancer Center, University of California San Francisco, San Francisco, CA; University of California San Francisco, San Francisco, CA; Cancer Stem Cells May Explain the Effectiveness of Dose-Dense Chemotherapy.

The Norton-Simon Hypothesis and Cancer Stem Cells: How Cancer Stem Cells May Explain the Effectiveness of Dose-Dense Chemotherapy.

The Norton-Simon Hypothesis and Cancer Stem Cells: How Cancer Stem Cells May Explain the Effectiveness of Dose-Dense Chemotherapy.

Materials and Methods: To evaluate TICs response to chemotherapy, mice with human breast tumor xenograft lines BCM-2665a and BCM-2147 were treated with vehicle, 10-, or 33-mg/kg docetaxel, and then tumors were collected for TIC assays and molecular analysis at both 48 and 72h after treatment. Using Affymetrix gene expression microarrays and reverse phase protein array (RPPA) analysis with 119 different validated antibodies, we identified pathways involved in regulation of TICs.

Results: According to flow cytometric analysis for TIC markers and mammosphere (MS) formation efficiency, BCM-2665 TICs were reduced by docetaxel treatment compared to vehicle-treated at 48h post 10 mg/kg docetaxel (4-fold decrease) and 48 and 72h post 33 mg/kg docetaxel compared to control (14- and 2-fold decrease, respectively). Although the BCM-2147 TICs did not significantly decrease at any time points or doses tested, they were clearly induced at 72h post-treatment compared to control. Additionally, BCM-2665 TICs were increased within 72h post 10 mg/kg docetaxel, indicating that these time points are ideal for chemotherapy. BCM-2147 TICs did not significantly decrease at any time points or doses tested, and then tumors were collected for TIC assays and molecular analysis at both 48 and 72h after treatment. Using Affymetrix gene expression microarrays and reverse phase protein array (RPPA) analysis with 119 different validated antibodies, we identified pathways involved in regulation of TICs.

Discussion: These in vivo studies and showed that there was a significant increase in ECM stiffness as the tissues transitioned from normal to an invasive lesion with the highest stiffness being located at the tumor edge (~2-4 folds greater). Intriguingly, we observed that ER positive tumors were substantially stiffer than ER positive tumors (50% increase in upper 10-percentile) and that there were tracks of ECM stiffness that correlated with oriented parallel collagen fibers and ECM birefringence. Moreover, we quantified a significant decrease in ECM stiffness following treatment (40% lower) with the most striking reduction in ECM tension being noted in ER negative patient tissues who demonstrated the most robust response. This study should lead to a deeper understanding of the nature of breast cancer stroma and its role in tumor phenotype and response to therapy.
findings are consistent with the Norton-Simon Hypothesis in that chemotherapy regimens given more frequently may in fact eliminate TICs, thereby explaining the proven increased effectiveness of dose-dense chemotherapy. Based on when TICs became chemoresistant, we are comparing dose-dense treatment (4 mg/kg docetaxel every 3 days) to a traditional single dose of 32 mg/kg, in an effort to eliminate the tumor cells that cause tumor recurrence. Furthermore, our analysis of gene expression at both the RNA and protein level implicated the immune cells as TICs inducers. Since our immunocompromised mice lack T- and B- cells but have active macrophages, macrophages are indicated as inducers of TICs. We are focusing our current efforts at identifying how immune cells activate TICs and thus enhance tumorigenesis.

P1-03-03
Adaptive Exploitation of Stromal Cell Metabolism by Tumor Cells.
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Tumor cells secrete cytokines to recruit and activate stromal cells in the tumor milieu leading to reciprocal paracrine support of tumor growth by stroma-derived growth factors. This is an important means by which tumors adapt their microenvironment to facilitate their growth. Indeed, breast cancer development and metastatic progression is highly dependent on stromal support, particularly from carcinoma associated fibroblasts (CAFs). As a result of aerobic glycolysis, tumor cells produce and secrete high levels of lactate, thought to be a toxic byproduct that needs to be extruded into the tumor milieu. Using CAFs generated by prolonged exposure to tumor conditioned medium, we have investigated the role that lactate may play in CAF-mediated support of tumor growth. In addition to extruding lactate as a byproduct of glycolysis, we suggest that tumor cells secrete it to recruit and subsequently exploit stromal cells to recycle lactate into utilisable metabolites, such as pyruvate, to fulfill metabolic demands of tumor cells. Our hypothesis is that lactate secreted by tumor cells is taken up by stromal cells and converted to metabolites critical for tumor growth and progression. Our studies indicate that (i) MDA-MB-231 breast cancer cells secrete significantly higher levels of lactate (3-fold more) under hypoxia, and that (ii) lactate recruits mesenchymal stem cells, the precursors of CAFs, towards tumor cells by activating signaling pathways to enhance migration. Lactate is transported by a family of proteins termed monocarboxylate transporters (MCTs); cells take up lactate via MCT1 and efflux it via MCT4. Expectedly, MDA-MB-231 breast carcinoma cells display low expression of MCT1 while exhibiting high expression of MCT4. However, CAFs show high expression of MCT1 while displaying low expression of MCT4, indicating that lactate extruded by the tumor cells is taken up by stromal cells. Our investigation further revealed that expression of lactate dehydrogenase B and pyruvate dehydrogenase are induced upon lactate exposure in CAFs, supporting the contention that the lactate taken up by stromal cells is metabolized. This implies that CAFs may be less glycolytic than MDA-MB-231s. Indeed the expression of pyruvate kinase isoform 2 (PKM2), which positively correlates with glycolysis, is greater in MDA-MB-231s than in CAFs, while CAFs express significantly more PKM1, the isoform that correlates with a more oxidative metabolic state. Finally, 13C NMR spectroscopic analyses indicate that 13C-lactate is indeed metabolized via the TCA cycle in stromal cells, further supporting our hypothesis. Thus, stromal fibroblasts in the tumor microenvironment have the capacity to take up tumor-secreted lactate and use it as an energy source. To our knowledge this is the first in vitro model system demonstrating tumor/stroma metabolic coupling by which tumor cells exploit stromal cells.

A better understanding of the molecular mechanisms governing metabolic cooperation within the tumor milieu will potentially identify new targets for therapeutic intervention.

P1-03-04
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Non-neoplastic tissues adjacent to tumor is known to influence the progression of the disease. We and others found that there are two types of microenvironment, one of these highly expresses genes associated with epithelial-to-mesenchymal transition (EMT). Tumor adjacent microenvironment (TAME) with wound responses have been observed in >90% of tumor-adjacent tissues, but EMT- characteristics seems to be less common and are observed in less than half of all patients. Our data suggest that the patients with EMT-TAME have worse breast cancer outcome, particularly among estrogen receptor positive patients. Thus, understanding the molecular cross talk between tumor cells and adjacent cells located in the TAME with EMT properties may identify targetable pathways that play a important role in breast cancer progression. Toward this end, we developed an in-vitro model system that can be used to probe paracrine signaling between cancer cells and adjacent cells undergoing EMT. We hypothesized that the presence of EMT signals in the TAME will alter the gene expression and phenotype of epithelial cells. We further hypothesized that the interactions between cancer cells and their TAME would be subtype dependent. Using cell line models of luminal and basal-like breast cancer, we performed cocultures with cancer cells and cell line models of EMT. The latter cell lines were HMLE cells transduced with either Snail or Twist, representing an intermediate state between an epithelial and a fully mesenchymal phenotype. This coculture system allows the study of heterotypic interactions between cancers and TAME cells with EMT characteristics. Gene expression, proliferation, migration, anchorage independent growth and cytokine production of different breast cancer and non-neoplastic cell lines were evaluated. We observed that coculture with models of EMT microenvironments altered the behavior of epithelial cells and that the gene expression changes were relevant to in-vivo in public datasets, showing correlations with both subtype and outcome. Cocultured cells acquired more migratory behavior and reduced expression of cellular adhesion genes, while simultaneously increasing anchorage independent growth and production of cytokines such as IL-6 and IL-8. Furthermore, we observed different response by luminal and basal-like breast cancer cells to the coculture condition, suggesting that the tumor-TAME interactions occur in a tumor subtype dependent fashion. These results reiterate the importance of the surrounding environment on cancer cell phenotypes and document that paracrine interactions with cells undergoing EMT may increase the aggressiveness of some cancers. Identification of specific signaling pathways that define interactions between specific breast cancer subtypes and their microenvironments
will lead to new insights regarding tumor biology and identify potential pathway-specific biomarkers and/or targets for prognosis and treatment of breast cancer.

P1-03-05
Fibrocystic Changes Have Different Age-Dependent Patterns in Benign, In Situ, and Invasive Breast Cancer Patients.
Bekhash A, Kovatchi AJ, Chen Y, Hooke JA, Kvecher L, Mural RJ, Shrider CD, Hu H. Windber Research Institute, Windber, PA; Walter Reed Army Medical Center, Washington, DC; MDR Global Systems LLC, Windber, PA

Background: Fibrocystic changes (FCCs) have been known to develop in the aging process. Breast cancers in younger and elderly women are known to have different characteristics. Less is known about alterations in the microenvironment for disease development in middle-aged women who undergo menopausal changes. The Walter Reed Army Medical Center, through the Clinical Breast Care Project, has enrolled over 2000 subjects undergoing a biopsy; all the pathology was reviewed by a single pathologist. These subjects provide an opportunity to study the age-dependent pattern of FCC across benign, in situ (IS) and invasive breast carcinoma (IBC) patient populations.

Methods: Subjects were enrolled following IRB-approved protocols. A total of 2060 subjects were identified for this study, including 1226 benign/atypical, 193 IS, and 641 IBC patients. Patients were divided into three age groups: <=45 years, 46-65 years, and >66 years. Chi-Square test in the SAS was used for statistical analysis across the age groups for the occurrence of FCCs. For IS and IBC patients, additional analysis was performed on high grade (grade 3) and non-high grade (grades 1 and 2) combined cancers. For IS, grade analysis was performed on ductal carcinoma (DCIS).

Results: As shown in the Table, FCC occurrence rate is significantly lower in younger patients in the benign population. In the IBC population, this rate is significantly higher in middle-aged patients. This higher rate is significant in the non-high grade patients but disappears in the high grade patients. In the IS population, the same trend exists that the FCC rate is higher in middle-aged patients. This trend is retained in non-high grade DCIS but again lost in high grade DCIS.

Discussion: The observation that FCC increases with age in the benign population, but is bell-shaped in IBC and IS populations, supports a hypothesis that physiological changes in the middle-aged women may alter the microenvironment for disease development. The observation that the bell-shaped pattern is due to non-high grade DCIS but not due to high-grade DCIS suggests that changes in the microenvironment contribute to breast disease development.

P1-03-06
Fibroadenomatoid Changes Have a Higher Occurrence Rate in Middle-Aged Benign Breast Disease Patients with the Trend Retained in Cancer Patients.
Bekhash A, Hooke JA, Chen Y, Kovatchi AJ, Kvecher L, Mural RJ, Shrider CD, Hu H. Windber Research Institute, Windber, PA; Walter Reed Army Medical Center, Washington, DC; MDR Global Systems LLC, Windber, PA

Background: Fibroadenoma (FA) is a common benign breast lesion known to have a high incidence rate in younger women. There are controversial reports whether FA elevates the risk of developing breast cancers. In clinical practice, FA may be surgically removed due to multiple reasons making it complicated to study its impact on the development of breast cancers that have a higher incidence rate in older women. Fibroadenomatoid change (FAC), also known as fibroadenomatous hyperplasia, is an uncommon lesion with histologic features similar to that of FA but lacking well-defined borders and usually discovered incidentally on breast biopsy specimens. FAC is not surgically targeted. The Walter Reed Army Medical Center, through the Clinical Breast Care Project, has enrolled over 2000 subjects undergoing a biopsy; all the pathology was reviewed by a single pathologist. These subjects provide an opportunity to study the age-dependent pattern of FAC in different patient populations.

Methods: Subjects were enrolled following IRB-approved protocols with data collected through two comprehensive questionnaires, a Core Questionnaire and a Pathology Checklist. A total of 1942 female subjects were identified for this study, including 1135 benign/atypical, 192 in situ, and 637 invasive cancer patients. Patients were divided into three age groups: <=45 years, 46-65 years, and >66 years. Chi-Square test in the SAS was used for statistical analysis.

Results: As shown in the table, FA occurrence rate decreases significantly with increasing age in benign disease patients. FAC, on the other hand, shows a significantly higher occurrence rate in middle-aged patients with benign findings, and this trend is retained in the invasive or in situ cancer populations. FAC rate is also significantly higher in patients with cancer (invasive, or invasive and in situ combined) compared to benign patients in each age group with p-values ranging from 0.0001 to 0.019 (not shown).

Discussion: Our preliminary results suggest that FAC occurs more often in middle-aged patients. It’s significantly lower occurrence in patients with benign findings may be partially explained by the fact that breast cancer patients undergo more extensive surgeries, thus providing more breast tissue for pathologic evaluation. Otherwise, the increased FAC rate may suggest its role as a risk factor for cancer development. Since FAC may be considered a miniature FA that is not surgically targeted, it may be used as a window for the study of FA on its impact in cancer development. Further study needs to be performed to explain why FA and FAC have different age-dependent patterns and whether FA or FAC is a risk factor for breast cancer development.

P1-03-07
Subtype-Specific Gene Expression Signatures in Peritumoral
Non-Neoplastic Breast Tissue.

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Tumors and histologically normal-tissue adjacent to cancer share many biological features with healing wounds. We previously described that the majority of cancer-adjacent normal (CN) tissues show activation of wound response gene expression signatures. However, CN tissue also shows considerable heterogeneity across patients. Therefore, to characterize interindividual variation in CN tissues, we performed unsupervised clustering on gene expression data from women with invasive breast cancer or ductal carcinoma in situ of the breast. Two distinct classes of CN tissue were identified, one of which showed gene expression consistent with epithelial-to-mesenchymal transition (EMT), a process that is well known to be activated in healing wounds. Activation of this signature was present in ~40% of cancer-adjacent normal specimens and it was also correlated with poor outcome among ER-positive patients. We next hypothesized that gene expression changes in cancer-adjacent normal tissue would be associated with breast cancer subtype and/or estrogen receptor status. To test this, we performed supervised analysis to identify subtype-associated gene expression profiles in CN. We observed that more aggressive tumor subtypes had distinct gene expression profiles in the adjacent normal tissue in supervised analysis. Specifically, Basal-like and luminal B tumors were characterized by reduced expression of claudins and cell adhesion genes, while also exhibiting higher expression of transcription factors associated with migration. These characteristic changes were validated in independent datasets by evaluating the ability of these signatures to predict subtype. It is possible to predict tumor subtype using just adjacent normal gene expression. Thus, our findings show that cancer-adjacent tissue may have value in identifying tumor subtype for cases where tumors are too small to allow genomic analyses. Furthermore, genomic field effects may have clinical implications if, as our data suggests, subtypes with higher local recurrence rates (e.g. basal-like and luminal B breast cancers) have widespread genomic alterations, even in non-neoplastic tissue. Widespread genomic changes may indicate a need for mastectomy rather than breast conserving therapy. Ongoing work is (1) assessing these signatures in association with relapse outcomes in the Polish Women’s Breast Cancer Study, where more than 10 years of follow up data have been accrued on more than 200 patients, and (2) using co-culture models systems to study paracrine communication between breast cancer cells and stroma according to breast cancer subtypes.

P1-03-08

Adipose Tissue in Breast Cancer: Not an Idle Bystander but an Active Participant in Breast Cancer Progression.


Background:

Adipose tissue is a dynamic organ that secretes a plethora of molecules called adipokines. In breast cancer we find a unique situation were adipokines play an important role. Thus, we hypothesize that adipocyte-derived factors influence breast cancer progression. Materials and methods:

Adipose tissue was collected from breast cancer patients undergoing a mastectomy. After macroscopic removal of blood vessels and connective tissue, the adipose tissue was carefully cut into 2-3mm3 pieces and were incubated in specific adipose-tissue culture medium. After 24h, the medium was collected and the quality was checked by determining the concentration of total proteins, leptin, adiponectin, TNFalpha and triglycerides. This conditioned medium of adipose tissue (CM AT) was used for in vitro experimentation with MCF-7 breast cancer cells.

Results:

Effect of AT on morphology and aggregation: when MCF-7 cells are grown in a culture flask, they tend to form round compact islands. Under influence of CM AT, the islands form sharp edges, the cells in an island can be counted individually and they show scattering. Importantly, despite the major changes in cellular morphology, CM AT removal rescued the compact island formation of MCF-7 cells. In the slow aggregation assay, cells treated with CM AT (and a subtherapeutic concentration of a neutralizing E-cadherin antibody) lost the ability to form compact aggregates. Furthermore, MCF-7 spheroids placed inside adipose tissue showed massive reorganization into an irregularly shaped mass.

Effect of AT on proliferation: starting from an equal number of cells and counting them every 2 days, it became clear that MCF-7 cells with CM AT had a higher rate of proliferation than MCF-7 cells in control medium. This stimulation of proliferation was confirmed by cell cycle analysis which revealed a doubling of cells in the G2/M phase, and western blot which showed an upregulation of cyclin A and cyclin E, both positive regulators of the cell cycle.

Effect of AT on invasion: a 24h collagen type I invasion assay revealed invasive characteristics of MCF-7 cells treated with CM AT while MCF-7 cells in control conditions are round and non-invasive. In contrast, a transwell collagen test over 14 days was not able to show MCF-7 cells invading the collagen gel under influence of CM AT. However, the growth pattern of MCF-7 cells on the collagen gel was clearly disorganised when compared with the control situation.

Conclusion:

These findings suggest that adipose tissue-derived factors exert a dramatic selective force on patterning, invasion and growth of MCF-7 breast cancer cells. Unraveling the mechanism behind these observations may provide vital information regarding the link between obesity and poor prognosis in postmenopausal breast cancer.

P1-03-09

Significance of FAP, SMA and CD31 Expression in the Stroma of Breast Cancer.

Tchou J, Satija C, Zhang P, Bi Y, Davuluri R, Chen H, Majumdar R, Mies C, Herlyn M, Pure E. Perelman School of Medicine at University of Pennsylvania, Philadelphia, PA; Perelman School of Medicine at University of Pennsylvania; Wistar Institute, Philadelphia

Breast cancer stroma heterogeneity has been demonstrated in various gene expression profile analyses. Whether there is any association between stroma heterogeneity and the molecular phenotype of breast cancer has yet to be established. Therefore, we performed immunohistochemical analyses (IHC) to evaluate the expression of the following stromal cell markers (fibroblast activation protein (FAP), smooth muscle actin (SMA), and CD31, an endothelial cell marker) in tumor tissues from a contemporary cohort of 52 patients comprising of all four molecular subtypes (luminal A (n=25); luminal B (n=2); Her2-neu (+) (n=5); and basal (n=20)). We hypothesize that stroma heterogeneity as reflected by the proportion of stromal cells staining (+) for FAP and SMA may correlate with their molecular epithelial phenotype. Furthermore, studying the distribution of these stromal cell markers in IHC sections may evaluate their spatial relationship.
with tumor cells, immune cells, and tumor microvasculature which may have strategic significance within the tumor/microenvironment.

Clinical characteristics of study cohort and stromal cell marker distribution in breast cancer tissue stratified by molecular subtypes

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Luminal A (n=25)</th>
<th>Luminal B (n=2)</th>
<th>HER2+ (n=5)</th>
<th>Basal (n=20)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age of diagnosis (years)</td>
<td>56.4±17.4</td>
<td>38.0±7.1</td>
<td>51.2±17.4</td>
<td>50.5±15.3</td>
<td>0.5959</td>
</tr>
<tr>
<td>Tumor size (cm)</td>
<td>3.2±1.6</td>
<td>2.5±0.3</td>
<td>8.1±6.8</td>
<td>15.7±1.7</td>
<td>0.0007</td>
</tr>
<tr>
<td>Tumor grade (mean mBRC score)</td>
<td>6.8±1.3</td>
<td>7.5±0.7</td>
<td>7.3±1.5</td>
<td>8.4±0.8</td>
<td>0.0008</td>
</tr>
<tr>
<td>No. positive nodes</td>
<td>4.2±5.3</td>
<td>4.0±1.5</td>
<td>2.5±3.8</td>
<td>2.6±5.5</td>
<td>0.5945</td>
</tr>
<tr>
<td>Fibroblast activation protein</td>
<td>mean % stromal cells (+)</td>
<td>86.2±12.7</td>
<td>50.0±0.0</td>
<td>92.0±8.4</td>
<td>84.4±14.8</td>
</tr>
<tr>
<td></td>
<td>mean intensity (range 3)</td>
<td>2.25±0.5</td>
<td>2.0±0.0</td>
<td>2.0±0.0</td>
<td>2.4±0.5</td>
</tr>
<tr>
<td>Smooth muscle actin</td>
<td>mean % stromal cells (+)</td>
<td>186.3±54.1</td>
<td>100.0±0.0</td>
<td>184.0±16.7</td>
<td>210.6±66.8</td>
</tr>
<tr>
<td></td>
<td>mean intensity (range 1-3)</td>
<td>30.4±33.1</td>
<td>5.0±0.0</td>
<td>52.0±17.9</td>
<td>24.3±27.7</td>
</tr>
<tr>
<td>Microvessel density</td>
<td>Mean no. CD31 (+) tumor forming microvessels per 20X magnification</td>
<td>50.8±8.4</td>
<td>27.0±2.8</td>
<td>28.3±5.5</td>
<td>25.2±11.0</td>
</tr>
</tbody>
</table>

As shown in Table 1, FAP is a more robust stromal cell marker staining 85±14% of stromal cells compared to SMA which stains only 28±29% of stromal cells (p<0.05). However, the distribution of FAP, SMA and microvessel density appears to be similar in all four subtypes. Multivariate analyses to correlate molecular subtype, tumor grade, tumor size, the no. of (+) nodes, and age with the % stromal cells staining (+) for FAP, SMA and CD31 yielded a significant correlation between the intensity of FAP(+) cells with tumor size, tumor grade, and the no. of positive nodes (p=0.00134, 0.0044, and 0.01141 respectively). We conclude that 1) stroma heterogeneity on IHC does not differ significantly across molecular subtypes; 2) FAP is a robust stromal cell marker; and 3) a higher FAP expression intensity on IHC may correlate with poor prognosis. Recent reports on the role of FAP in promoting tumor growth plus the abundance of FAP expression in breast cancer stroma underscore a significant role of FAP in breast cancer.

P1-04-01
The Mechanism of Anti-Breast Cancer TICs Effect of Pyrvinium Pamoate Is through WNT/beta-Catenin Signaling.
Xu W, Debeb B, de Lacerda A, Li L, Larson R, Reuben J, Ueno N, Woodward W. UT MD Anderson Cancer Center, Houston, TX

We have previously shown that pyrvinium pamoate can decrease breast cancer TICs in vitro and shrink the tumor size in vivo. Although pyrvinium pamoate has been shown to target beta-catenin through activating CK-1alpha in a vitro model, the mechanism of its anti-breast cancer TICs effect is unknown. Herein, we use a constitutively active WNT/beta-catenin signaling construct EBETAP (ref) to determine if the anti-TIC effect of pyrvinium pamoate is through WNT/beta-catenin signaling. Using aldefluor expression and mammosphere formation efficiency as TIC surrogate assays, we found that TICs of SUM-159 transfected with EBETAP construct are resistant to pyrvinium pamoate treatment compared to control cells. Moreover, microarray analysis reveals a series of genes and signaling downstream of WNT-beta-catenin were down-regulated in SUM-159 cells treated with pyrvinium pamoate. In summary, mechanism of anti-breast cancer TICs effect of pyrvinium pamoate is through WNT/beta-catenin signaling.

P1-04-02
In Vitro Qualification and Quantification of Murine Mammary Stem/Progenitor Cells.
Dong Q, Wang D, Bandyopadhyay A, Liu Z, Yu L, Gao H, Moncada K, Huang C, Walter CA, Sun L-Z. University of Texas Health Science Center, San Antonio, TX; Wenzhou Medical College, Wenzhou, China

Increasing evidence suggest that tumors with a cell origin of more basal or stem-like sources are often highly metastatic and associated with poor prognosis. Identification of the cell of origin thus has important implications for development of new preventive and therapeutic approaches. To identifying the cells of origin of various breast cancers, understanding the normal cellular hierarchy within the breast tissue is an important prerequisite. However, current understanding of normal mammmary stem/progenitor cell is limited due to the lack of a robust in vitro assay. The newly developed cell surface markers (CD24+CD49dim+) can only be used for low percent of enrichment. The only gold standard assay for functional evaluation of stem cell property is the in vivo transplantation of cleared mammary fat pad assay, yet this assay is expensive, time consuming, and requires highly trained skills, and thus cannot be used routinely for experiments. The goal of our study is thus to develop a robust in vitro assay to qualify and quantify murine mammary stem/progenitor cells. Our rationale lies on our recent findings of in vitro study of different fractions of murine stem/progenitor cells. When we use isolated primary epithelial cells for in vitro mammosphere formation, we found that stem cell enriched fraction and progenitor cell enriched fraction both formed very small mammospheres (≤ 50 µm) and these spheres appear to be of clonal origin (one cell gives rise to one sphere). When we plated these small spheres into 3D extracellular matrix for colony formation, however, they gave rise to two distinct structures: stem-enriched fraction generated predominately solid structure while progenitor-enriched fraction is dominated by hollow structures. Previous studies have linked the solid structure to the basal/myoepithelial lineage and the hollow structure to the luminal lineage. We thus suspected that the small mammospheres derived from the stem or progenitor enriched fractions could be originated from single stem or progenitor cell, and the number of spheres could indicate the number of original stem/progenitor cells within these enriched fractions. Subsequent in vivo transplantation with single sphere or single solid structure cultured in 3D extracellular matrix derived from green fluorescent protein transgenic mice proved that single sphere or 3D solid structure can repopulate the gland-free mammary fat pad. To conclude, the in vitro mammosphere formation in combination with subsequent differentiation in 3D extracellular matrix can be used as a robust in vitro assay for qualification and quantification of murine mammary stem/progenitor cells. This in vitro assay will greatly facilitate our understanding of genes regulate stem cell self-renewal, proliferation, differentiation as well as mechanisms keeping them at quiescent state within the niche.

P1-04-03
The Effect of Survivin Downregulation on Radiosensitization of Breast Cancer Cell Lines Grown under Adherent and Stem Cell Promoting Culture Conditions.
Debeb BG, Larson R, Xu W, Lacerda L, Reuben JM, Buchholz TA, Ueno NT, Woodward WA, Morgan Welsh Inflammatory Breast Cancer Clinic and Research Group, The University of Texas M.D. Anderson Cancer Center, Houston, TX

Survivin, the smallest member of the inhibitor of apoptosis protein (IAP) family, is a bifunctional protein that has been implicated in both...
control of cell division and inhibition of apoptosis. Survivin has been shown to be involved in radiation resistance of various cancer types and its expression is increased by sublethal doses of irradiation in both differentiated and cancer stem cell (CSC) population. However, it is unknown whether suppression of survivin radiosensitizes the CSC population or diminishes its self renewal ability. Herein, we cloned the survivin dominant-negative mutant lacking a phosphorylation site (T34A) (Mesri et al., 2001 JCI) into the lentiviral LeGo vectors and assessed mammosphere formation and radiosensitization in MCF7, SUM149 and SUM159 cell lines grown under adherent or stem cell promoting conditions. We found an induction of survivin by western blotting in the dominant negative mutant (T34A)-transfected cell lines. Moreover, we observed a higher than two-fold increase in the Sub-G1 population as well as an increased Caspase 3 activity in the T34A-transfected SUM149 and SUM159 cells compared to control cells indicating an anti-apoptotic function of survivin. We also found that T34A-transfected cells showed a 1.5 to 2-fold decrease in the number of mammospheres compared to the control-transfected cells. Furthermore, T34A-transfected cells showed radiosensitization in adherent cells from SUM149 and SUM159 cells but no effect was observed in MCF7 cells. However, no radiosensitization was observed in stem cell promoting culture conditions with increasing doses of radiation in all tested cell lines. This indicates that the widely used standard clonogenic assays do not optimally select anti-CSC agents and that targeted therapies should be specifically tested for their activity against the CSC population. Further functional endpoint studies will be conducted to validate the in vitro findings.

P1-04-04
Focal Adhesion Kinase Plays a Major Role in the Regulation of Human Ductal Carcinoma In Situ (DCIS) Stem Cell Activity.
Williams KE, Bundre NJ, Farnie G. University of Manchester, Paterson Institute for Cancer Research, Manchester, United Kingdom; University Hospital of South Manchester, Manchester, United Kingdom

Focal Adhesion Kinase (FAK) is a non-receptor tyrosine kinase that has been implicated in the regulation of normal mammary stem cells and cancer stem cells (CSCs) within mouse models. FAK is frequently overexpressed in human breast cancers but its role in human CSCs is not known. We aimed to investigate the role of FAK in human ductal carcinoma in situ (DCIS) stem cells (SCs). We have used PF573228 to inhibit FAK activity in SUM225 (HER2-overexpressing) and MCF10DCIS.com (HER2-normal) DCIS cell lines. PF573228 inhibits the autophosphorylation of FAK at tyrosine 397 causing inactivation. Western blot analysis confirmed PF573228 (0.1-5µM) caused a dose dependent inhibition of FAK activity in both cell lines. The in vitro mammosphere colony assay was used to measure CSC activity in both DCIS cell lines and primary DCIS samples (n=5) (LREC/01/012). Mammosphere-forming efficiency (MFE) was calculated as percentage colony formation (>60µm) in the presence or absence of PF573228 (0.1-5µM). MFE was reduced in both the SUM225 cell line (p=0.02) and in the MCF10DCIS.com cell line (p=0.04) by PF573228 at a dose concentration of ≥0.1µM (see Table 1). PF573228 (1µM) also reduced mammosphere formation in human primary DCIS cells (n=5) by 18.1±5.75% when compared to control (p=0.03).

Table One Shows the Primary Generation Mammosphere Forming Efficiency (MFE) in the DCIS cell lines.

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Dose Concentration of PF573228 (µM)</th>
<th>MFE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SUM225</td>
<td>0</td>
<td>0.71±0.05</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>0.46±0.11</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>0.38±0.02*</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>0.28±0.04*</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>0.31±0.13*</td>
</tr>
<tr>
<td>MCF10DCIS.com</td>
<td>0</td>
<td>0.84±0.04</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>0.79±0.03</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>0.61±0.10</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>0.44±0.04</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>0.48±0.01</td>
</tr>
</tbody>
</table>

Note: (a) p=0.02, (b) p=0.04 and (c) p<0.0001 compared to control, Mann Whitney U, 2-tailed test.
To measure the effect of PF573228 on DCIS SC self-renewal capacity we performed secondary generation mammosphere colony assays. Primary generation mammamospheres that had been treated with PF573228 (0.1-5µM) were dissociated into a single cell suspension and reseeded out into mammosphere culture conditions with no additional PF573228. The mammosphere regeneration ratio (MRR) was calculated as the proportion of secondary mammamospheres formed relative to the number of primary mammamospheres. MRR was reduced in both the SUM225 (p=0.005) and MCF10DCIS.com (p=0.001) cell lines after treatment with ≥0.5µM or ≥1.0µM PF573228 respectively (see Table 2). Preliminary data in human primary DCIS cells (n=1) also shows a reduction in MRR, from 0.56 to 0.14 with 0.5µM PF573228. These results suggest that CSC self-renewal capacity is reduced by FAK inhibition.

Table Two Shows the Secondary Generation Mammosphere Regeneration Ratio (MRR) in the DCIS cell lines.

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Dose Concentration of PF573228 (µM) in primary culture only</th>
<th>MRR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SUM225</td>
<td>0</td>
<td>0.71±0.05</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>0.46±0.11</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
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<td></td>
<td>1.0</td>
<td>0.44±0.04</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>0.48±0.01</td>
</tr>
</tbody>
</table>

Note: (a) p=0.005, (b) p=0.004, (c) p=0.005 and (d) p<0.0001 compared to control, unpaired, 2-tailed t-test.
We conclude that FAK signalling is important in human DCIS SC activity measured by mammosphere growth and regeneration. Targeting FAK in the treatment of DCIS may reduce disease recurrence and improve patient outcome.

P1-04-05
Honig A, Diessner J, Dietl J, Wischniessen J. University of Wuerzburg, Wuerzburg, Germany

Trastuzumab (Herceptin®) has significantly improved the overall survival of patients with HER2-positive breast cancer. The efficacy of this treatment depends significantly on antibody-dependent cell mediated cytotoxicity (ADCC). However, while trastuzumab can direct NK cells towards tumor cells and thereby promote tumor cell lysis, spheroid-forming tumor-initiating cells are largely spared. Consequently, populations of surviving cells showed greatly enhanced clonogenicity in vitro and tumorigenicity in vivo. Low HER2 levels and reduced expression of activating NK cell ligands provide likely explanations for the escape of these cells from HER2-directed NK cell-dependent therapies. Moreover, recent data show that unproductive interactions between immune cells and cancer cells can induce a process called “epithelial-to-mesenchymal transition” (EMT) which enables previously differentiated cancer cells to acquire stem cell-like properties as evidenced by a CD44hi/CD24low breast cancer stem cell signature and aldehyde dehydrogenase positivity. We thus investigated whether new HER2-specific antibodies could enhance NK-cell mediated target cell lysis or inhibit the induction of tumor stem cells. The antibody pertuzumab also binds to the HER2-receptor. It inhibits homo- and heterodimerization of the HER2 receptor with HER3 receptors. We were able to show that the combination of trastuzumab and pertuzumab leads to increased antibody-binding on breast cancer cells and breast cancer stem cells, resulting in enhanced ADCC. This correlates with the clinically observed synergy between trastuzumab and pertuzumab as observed for example in the...
Neosphere trial, a neoadjuvant study for HER2-positive breast cancer. Trastuzumab emtansine (T-DM1) is an antibody-drug conjugate which combines trastuzumab with the tubulin inhibitor maytansine. Interestingly, treatment with T-DM1 largely prevented the NK cell-dependent induction of the EMT markers Twist and Snail and the characteristic downregulation of E-cadherin. Thus, T-DM1 apparently interferes with EMT and may hence prevent the re-induction of the stem cell phenotype. In line with the presumed effect against cancer stem cells, T-DM1 significantly attenuated the clonogenic potential of FACS-sorted trastuzumab-unresponsive cancer stem cells. Accordingly, our data show that these new therapeutics may be more effective against cancer stem cells and thereby promise a more sustained treatment of HER2-positive breast cancer.

P1-04-06
Ionizing Radiation Reprograms Non-Tumorigenic Cancer Cells into Cancer Stem Cells.
Chann L, Vlasi E, Lorenza DD, Dekmezian C, Pajonk F. University of California, Los Angeles, CA
Breast cancers are thought to be organized hierarchically with a small number of breast cancer stem cells (BCSCs) able to re-grow a tumor while their progeny lack this feature. BCSCs in breast cancer have been found to be relatively resistant to radiation and several groups reported enrichment for BCSCs when breast cancers are subjected to classical anticancer treatment. Differentiation of BCSCs is thought to be unidirectional but an alternative model assumes that stemness can be obtained by clonal evolution. In this study, we quantified the number of BCSCs surviving after radiation treatment. We compared the number of surviving BCSCs to the expected number and found an increase in BCSCs after irradiation that could not be explained by current models. We propose that radiation induces a BCSC phenotype in previously non-BCSCs and show that this transition is Notch-dependent and coincided with up-regulation of the transcription factors Oct4, Sox-2, Nanog, and Klf4.

P1-04-07
Poly (ADP-Ribose) Polymerase-1 (PARP-1) Is Overexpressed in Human Breast Cancer Stem Cells: Results from a Proteomic-Based Approach.
Gilabert M, Ginestier C, Audebert S, Pophillat M, Toirnon Y, Birnbaum D, Borg J-P, Charafe-Jauffret E, Gonçalves A. Institut Paoli-Calmettes, Marseille, France; U981 INSERM, Marseille, France; Université de la Méditerranée, Marseille, France; Centre d’Immunologie Marseille-Luminy, Marseille, France
Introduction: Cancer stem cells (CSC) have been increasingly recognized as playing a major role in various fields of breast tumor biology including carcinogenesis, metastasis and resistance to cytotoxic drugs and radiotherapy. Identification of protein biomarkers associated with breast CSC may help understanding CSC biology as well as identifying novel diagnostics and specific therapeutic targets. 2-D Fluorescence Difference Gel Electrophoresis (2-D DIGE) is a method that labels protein samples with fluorescent dyes before 2-D electrophoresis, enabling accurate analysis of differences in protein abundance between samples. Methods: Using flow cytometry-based ALDEFLUOR assay (ALD), we isolated CSC-enriched (ALD-positive) and non CSC-enriched (ALD-negative) cell populations of MDA-MB-453, a human breast cancer cell line. Total proteins were extracted from both fractions using urea-based buffer and subjected to 2D-DIGE. Differentially expressed spots were excised, proteins were gel-extracted, digested and identified using MALDI-TOF MS (Ultraflex, Brucker Daltonics, Billerica, USA).

Results: 2D-DIGE revealed differential expression of various protein spots between ALD-positive and ALD-negative MDA-MB-453 cells. MALDI-TOF MS analysis allowed identification of 11 differentially expressed proteins, among which 7 were down-regulated and 4 were up-regulated in ALD-positive MDA-MB-453 cells, including Poly (ADP-ribose) polymerase-1 (PARP-1). Overexpression of PARP-1 in MDA-MB-453 cells was further confirmed by western blot using specific monoclonal antibody and such an observation was extended to 4 additional human breast cancer cell lines including HCC1937, MDA-MB-436, SUM149 and SUM159. These 5 human breast cancer cells were found to display a limited short-term sensitivity (within the micromolar range) to olaparib, a specific PARP-1 inhibitor. However, a negative correlation was found between the level of overexpression and the ability of olaparib to inhibit the growth of ALD-positive cells. Conclusion: A proteomic-based approach revealed that PARP-1 was up-regulated in ALD-positive, CSC-enriched from various human breast cancer cell lines. Such an overexpression may contribute to clinical resistance to PARP-inhibitors.

P1-04-08
Distribution of ALDH1 Positive Stem Cells in Benign Mammary Tissue from Women with and without Breast Cancer.
Isfoss BL, Holmqvist B, Alm P, Olsson H. Telemark Hospital, Skien, Norway; Lund University, Lund, Sweden
Background and Aims. ALDH1 in female breast tissue has been linked to stem cells, but little is known about the benign cellular organisation in situ. We investigated the distibution of ALDH1 immunoreactive (ALDH1+) cells in histomorphologically benign breast tissue from 28 women with or without breast cancer.

Methods and Results. ALDH1+ cells were detected in benign tissue of women age 20 - 72, located most commonly within the luminal and intermediate ductular levels and in the stroma. ALDH1+ cell populations and Ki-67+ cell populations were present in separate ductules, both cell types rarely showing epithelial differentiation. ALDH1+ cells were non-reactive to Ki-67 and oestrogen receptor. Stromal round/oval ALDH1+ non-leukocyte cells in both age groups expressed contractile protein. There was a lower concentration of luminal and intermediate ductular ALDH1+ cells in post-menopausal women than pre-menopausal women, lower in cancer than non-cancer patients, and higher in women on exogenous hormones.

Conclusions. The study provides further evidence for the stem cell character of ALDH1+ cells, here in benign breast tissue of cancer and non-cancer women throughout non-lactating adult life, and contributes evidence of benign stromal ALDH1+ cells. The distribution of ductular ALDH1+ stem cells appears to be influenced by hormonal status.

P1-04-09
Biphasic Effects of Docetaxel and Hedgehog Signaling Antagonists on Breast Cancer Tumor-Initiating Cells In Vivo.
Zhang X, Moraes RC, Landis MM, Wu M-F, Hilsenbeck SG, Cairo MM, Tofigar R, Chang JC, Lewis MT. Baylor College of Medicine, Houston, TX; Karolinska Institutet, Novum, Sweden
Recent data suggest the existence of a subset of breast cancer cells variously termed cancer stem cells, tumor-initiating, or tumor-propagating cells, that are capable of self-renewal and of regenerating tumors upon transplantation that are biologically consistent with the tumor of origin. These cells appear to be intrinsically resistant
RESULTS: Our results show that PELP1 uniquely recognizes histones modified by arginine dimethylation, arginine citrullination and lysine dimethylation. Phosphorylation of residues adjacent to a methyl modification affects the ability of PELP1 to recognize histone methylation. Using various deletions of PELP1 peptides, we have found that PELP1 acts as a module for recognition of a specific histone modification through the carboxyl-terminal glutamic acid rich region. Reporter gene assays showed that PELP1 functionally interacts with arginine methyltransferases including CARM1 and PRMT6, both shown to be dysregulated in human cancers, and synergistically enhances ER-transactivation. Chromatin immunoprecipitation assays revealed that PELP1 has the potential to alter histone H3 arginine methylation status at ER target gene promoters. PELP1 knockdown via PELP1-siRNA liposomes using xenograft based assays resulted in decreased arginine dimethylation with concomitant reduction in tumor volume.

Conclusions: Our findings suggest that PELP1 is a reader of histone methyl modifications and deregulation of PELP1 may have implications on tumor proliferation via epigenetic alterations at ER target promoter. Targeting these epigenetic alterations through inhibition of PELP1 and the arginine methyltransferases could be a promising cancer therapeutic. This study was funded by CPRIT pre-doctoral fellowship grant and Komen KG090447.

P1-05-02
Epigenetic Regulation by Alcohol Reactivates Estrogen Receptor alpha in Estrogen Receptor alpha-Negative Cells.

Wong AW, Nunez N. University of Texas at Austin, Austin, TX

Introduction: Alcohol consumption is an established risk factor for breast cancer development and contributes to mammary tumorigenesis through the regulation of estrogen receptor alpha (ERα) expression. Previously, we reported that alcohol consumption in the MMTV- neu ERα-negative mouse model of human breast cancer resulted in significantly increased ERα expression compared to non-alcohol consuming mice. Thus, our results indicated that alcohol consumption led to reexpression of ERα in an ERα-negative mouse model. Several lines of evidence suggest that ERα expression in ERα-negative cancer cells is inhibited through epigenetic mechanisms. In this study, we examine the role of alcohol on ERα reactivation through changes in histone modifications, DNA methylation, and recruitment of transcriptional regulation complexes on the ERα promoter, in vitro. Methods: ERα-negative MDA-MB-231 human breast cancer cells were treated with 0.1%, 0.2% and 0.5% v/v ethanol in culture media for 24 h. Chromatin immunoprecipitation (ChIP) assays were used to examine the enrichment of active and inactive markers of chromatin on the ERα promoter following alcohol treatment. DNA methylation at three ERα promoter CpG dinucleotide sites was assessed following alcohol treatment using methyl-sensitive restriction enzyme (MSRE)-qPCR. ChIP assays are used to determine the binding of the transcriptional regulation complexes, pRB2/p130-E2F4/5-HDAC1-SUV39H1-MSRE-qPCR, to the ERα promoter following alcohol treatment. MCF-7 human breast cancer cells served as an ERα-positive control.

Results: We report that histone modifications were affected by alcohol treatment in a dose-dependent manner. Enrichment levels of all five chromatin markers tested, which includes markers of active and inactive transcription, were significantly altered as a result of alcohol treatment (p<0.05). We also found significantly decreased DNA methylation at three ERα promoter CpG sites as a result of alcohol treatment (p<0.05). Experiments testing the recruitment of the
transcriptional regulation complexes, pRb2/p130-E2F4/5-HDAC1-SUV39H1-p300 and pRb2/p130-E2F4/5-HDAC1-SUV39H1-DNMT1, are currently ongoing. Discussion: ERα expression has been linked to mammary carcinogenesis and clinical outcome in breast cancer patients. However, approximately one out of three breast cancers lack ERα expression at the time of diagnosis and many breast cancers lose ERα expression during the course of tumor progression. Thus, understanding the regulation of ERα expression in breast cancer cells is critical and results from this study will facilitate the development of novel therapeutic strategies and treatment options for hormone-resistant breast cancer.

P1-05-03
Relationship between Polycomb Repressive Complex EZH2/CBX7, Large Non-Coding RNA ANRIL and Stem Cells Biomarkers in Triple Negative Breast Carcinomas: Important Role in Carcinogenesis through an Epigenetic Silencing Process and New Clues for Targeted Therapies.

Background: Epigenetic deregulation and carcinogenesis are intimately connected and gene silencing is a major consequence of epigenetic modifications. Polycomb group proteins (PcG) play important roles by inhibiting chromatin remodeling and transcription, silencing tumor suppressor genes, regulating stem cells and interconnecting with Wnt/beta-catenin, TGF-beta and Sonic-Hedgehog pathways. EZH2 is a transcriptional repressor and acts mainly as an oncogenic silencer gene involved in cell cycle regulation. Large non coding RNA are major regulators of oncogenic and oncosuppressive pathways at epigenetic, transcriptional and post-transcriptional levels. Recent studies have showed that (i) EZH2 overexpression is associated with cancer progression and acquisition of stem cell properties and (ii) disruption of ANRIL/CBX7 interactions repress the INK4b/ARF/INK4a locus. The aim of the study was to analyze PRC complexes, large non-coding RNA ANRIL and downstream signaling targets in triple negative breast carcinomas (TNC).

Design: By using real time quantitative RT-PCR in a series of 432 invasive breast ductal carcinomas (IDC), we quantified gene expression levels of the large non coding RNA ANRIL, PcG (EZH2, CBX7, SUZ12), stem cells markers (ALDH1, CD133, ASCL1) and oncosuppressor genes (p15, p16 and p19). Immunohistochemistry (IHC) was performed using EZH2, CBX7, ALDH1 and b-catenin Abs. We compared mRNA expression levels and IHC intensity score in TNC with non TNC and normal breast parenchyma.

Results: EZH2 overexpression was respectively found by RT-PCR and IHC in the TNC subgroup of IDC. We observed variable mRNA expression levels of SUZ12, CBX7, ANRIL, ALDH1, CD133, ASCL1, p15, p16 and p19. IHC showed EZH2 and CBX7 nuclear staining. Mutual positive correlations were found between EZH2, CBX7, ALDH1, ASCL1 and CCND1 (p<0.05). Conclusion: Polycomb EZH2/CBX7 and the large non coding RNA ANRIL are epigenetic transcriptional repressors overexpressed in the TNC subgroup of IDC. Their implication in the process of gene silencing (p16, p15, p19), activation of oncogenic pathways (Wnt, TGFβ) and tumor stem cells self-renewal may explain several hallmarks of TNC and open new avenues to target this aggressive subgroup.

P1-05-04
Distinct Patterns of Promoter CpG Island Methylation of Breast Cancer Subtype Are Associated with Different Stem Cell Phenotype.
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Background: Although DNA methylation profiles in breast cancer have been connected to breast cancer molecular subtype, there have been no studies of the association of DNA methylation with stem cell phenotype. This study was designed to evaluate promoter CpG island methylation of 15 genes with regard to breast cancer subtype and to investigate whether the patterns of CpG island methylation in each subtype are associated with cancer stem cell phenotype represented by CD44+/CD24- or ALDH1 expression.

Methods: We performed MethyLight analysis for the methylation status of 15 promoter CpG island loci involved in breast cancer progression (APC, DLEC1, GRIN2B, GSTP1, HOXA1, HOXA10, IGF2, MT1G, RARB, RASSF1A, RUNX3, SCD3, SUZ12, TMEM124, and TMEFF2) and determined cancer stem cell phenotype by CD44+/CD24- and ALDH1+ immunohistochemistry in 36 luminal A, 33 luminal B, 30 luminal-HER2, 40 HER2 enriched, and 40 basal-like subtypes of breast cancer.

Results: The number of CpG island loci methylated was significantly different among subtypes and it was highest in luminal-HER2 subtype and lowest in basal-like subtype. Methylation frequencies and levels in 12 out of the 15 genes were significantly different among all subtypes and basal-like subtype showed significantly lower methylation frequencies and levels in nine genes, compared to luminal A, luminal B, HER2 enriched, and luminal-HER2 subtypes. CD44+/CD24- or ALDH1+ putative stem cell populations were most enriched in basal-like subtype. The methylation of promoter CpG islands was significantly lower in CD44+/CD24- cell (+) tumors, compared to CD44+/CD24- cell (-) tumors, and even within the basal-like subtype, ALDH1 (+) tumors also had significantly lower methylation, compared to ALDH1 (-) tumors.

Conclusions: Our findings showed that promoter CpG island methylation was significantly different according to breast cancer subtype and stem cell phenotype of tumor, suggesting that breast cancers have different methylation patterns according to molecular subtypes and it is associated with stem cell phenotypes of the tumor.

P1-05-05
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Background: Gene expression is stringently controlled under physiological conditions by epigenetic mechanisms, including the specific methylation of cytosine residues within CpG dinucleotides and orchestrated adjustments in the histone dependent organisation of chromatin. The organisation of DNA within the chromatin template depends upon highly conserved histone proteins, the properties of which continue to exceed the simplistic packaging role originally assigned to them. Histone modifier enzymes impart a dynamic histone code and specific permutations have significant implications for
chromatin topology and the functional configuration of promoters. This study follows our initial report describing potential tumour suppressor function associated with the histone methyltransferase SETD2 in human breast cancer. The objective was to evaluate the expression profiles of sixteen additional histone modifier genes in women with primary operable breast cancer within a well annotated cohort with extended follow-up.

Methods: Primary breast cancer tissues (n=127) and adjacent benign/normal tissues (n=33) underwent RNA extraction and reverse transcription. The transcript levels of histone modifier genes were evaluated using real-time quantitative PCR, these included: histone acetyltransferases (CREBBP), Class 1 (HDAC1 and HDAC2), II (HDAC5) and III (SIRT1) histone deacetylases and histone methyltransferases (SUVR9H1 and SUVR9H2) amongst others. Transcript levels were analysed against a range of clinico-pathological variables, including: tumour size, grade, nodal involvement, histological subtype, receptor status, TNM stage, Nottingham Prognostic Index, disease free and overall survival over a 10 year follow-up period.

Results: Transcript levels of the histone modifier genes in breast cancer tissues differed significantly from non-malignant samples (HDAC5, HDAC1, KDM4A and KDM6A). Amongst breast cancers, significant differences in transcript levels were associated with established pathological parameters and prognostic indices: tumour grade (KAT5, HDAC1, KDM4A, SUVR9H1 and KDM6A), receptor status (KAT5, SMYD3 and KDM1A), histological type (KAT5, KDM5C, MYST1, KDM4A and MLL), TNM stage (SUVR9H1, KAT2B, KDM1A, KDM4A, KDM5C, MYST1, HDAC5 and KAT5), Nottingham Prognostic Index (KDM5C, MLL, MYST1 and SMYD3), disease free survival (SUVR9H1, SMYD3, HDAC5, KDM6A, HDAC1, KDM1A, KDM4A, MYST1, KDM5C, KAT5 and MLL) and overall survival (MYST1). Interestingly, significant correlations were also identified between the differential expression profiles of particular histone modifying genes.

Conclusion: The expression profiles of histone modifier genes differ significantly between breast cancer tissues and normal/benign samples. Particular expression profiles in breast cancer are significantly associated with established pathological parameters, prognostic indices, disease free and overall survival. The biological significance and clinical relevance of altered expression of specific histone modifier genes and particular permutations of misexpression remain to be fully elucidated and further study is warranted. Epigenetic signatures derived from histone modifier genes may offer utility as biomarkers and histone modifier enzymes have potential for targeted therapeutic strategies.

P1-05-06
Hypermethylation 14-3-3- Sigma Promoter as a Biomarker to Screening for Metastasis and Potential Prognostic Factor in Breast Cancer Patients.

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Background: 14-3-3sigma is an epithelial marker whose expression is induced by DNA damage through a p53-dependent pathway. 14-3-3sigma functions sequesters cyclin B1-CDC2 complexes outside the nucleus and thereby contributes to a G2 arrest. Epigenetic silencing by CpG methylation, p53 inactivation, and proteasome-dependent proteolysis leads to loss of 14-3-3sigma and is often observed in precancerous lesions and likely to be causally linked to the onset of cancer.

Objective: To correlation methylation levels of promoter 14-3-3 sigma with association prognostic factors in breast cancer and their correlation with phenotype luminal.

Material and Methods: This is a prospective study we quantified methylation levels of promoter 14-3-3 sigma gene in 107 women with breast cancer and 108 control subjects by Real Time QMS-PCR SYBR green (methylation-specific PCR) and analyzed association with prognostics factor in breast cancer.

Results: Median age was 58 years (32-88); 69% were postmenopausal women. Nodal involvement N0; 63%,N1;30%,N2;7%, tumor size (T1;58%,T2;35%,T3;4%,T4;4%) and grade G1;20%,G2;37%,G3;30%). Significant differences between breast cancer patients (pts) and healthy controls in relative serum levels of methylation gene promoters 14-3-3s (p=0.0047) were detected. Presence of methylated 14-3-3-σ in serum of breast cancer patients was associated with T3-4 stage (OMS) (p<0.05) and nodal positive status (p<0.05). With a median follow up 6 years we saw more probability of developing distant metastasis in patients with methylation 14-3-3 sigma (p<0.05).

Conclusions: Hypermethylation of the 14-3-3 sigma promoter is an early and frequent event in breast neoplastic transformation, leading to the suggestion that silencing of 14-3-3 sigma may be an important event in tumor progression and particularly in breast carcinogenesis. This study identifies the presence of variations in global levels of methylation promoters genes in patients breast cancer with different phenotype classes and shows that these differences have clinical significance. Perhaps in the detection of CpG methylation of 14-3-3sigma may be used for diagnostic and prognostic purposes.

P1-06-01
Withdrawn by Author

P1-06-02
Correlation between Gene Variants in CYP19 (Aromatase) and TCL1A with Disease and Tolerability Endpoints in the ATAC Trial.

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Aim: To assess whether candidate polymorphisms in CYP19 (aromatase) and TCL1A were associated with recurrence, musculoskeletal symptoms (MSKs) or hot-flushes in primary estrogen receptor-positive breast cancer in postmenopausal women treated with tamoxifen or anastrozole.

Background. Recently, variants rs10459592 and rs4775936 in CYP19 (aromatase) and TCL1A were associated with recurrence, MSKs or hot-flushes in primary estrogen receptor-positive breast cancer in postmenopausal women treated with tamoxifen or anastrozole.

Methods: This is a prospective randomized double-blind placebo-controlled trial that compared the adjuvant use of anastrozole versus tamoxifen for 5 years. The trial’s detailed efficacy and safety data, long term (10-year) follow-up and high event rate make it an ideal setting for pharmacogenetic single nucleotide polymorphism (SNP)
validation studies. Here we report our findings testing for correlations with 3 SNPs in CYP19 and TCL1A with clinical outcomes including any disease recurrence, distant recurrence, MSKs, and hot-flushes. 

Methods: Genotypes were determined using ABI Taqman assays without knowledge of the patient’s treatment assignment or outcomes. Hazard ratios (HR) and corresponding 95% confidence intervals (CI) were estimated by the proportional hazards regression model and were adjusted for: age, tumor size, grade, and nodal status (for survival analyses) and age, hormone replacement therapy, body mass index, and smoking (for toxicity analyses).

Results: 807 patients were genotyped for CYP19 SNPs rs10459592 and rs4775936 and 842 for TCL1A SNP rs11849538. The rs10459592 genotype frequencies were 16.6% Wt/Wt, 51.5% Wt/Vt, and 32% Vt/Vt. There was a trend towards decreased recurrence in Vt/Vt and Vt/Wt compared to Wt/Wt (HR=0.77 [0.50-1.22] and 0.68 [0.42-1.09], p[ trend]=0.1), similar for both anastrozole- and tamoxifen-treated patients and also similar for distant recurrence. For the same SNP, there was a trend toward more MSKs in Vt/Vt and Vt/Wt compared to Wt/Wt (HR=1.22 [0.80-1.88] and 1.47 [0.94-2.36], p[ trend]=0.08), primarily due to the effect in the tamoxifen cohort (HR=1.88 [0.89-3.99], p[ trend]=0.1). The rs4775936 genotype frequencies were 26.0% Wt/Wt, 52.1% Wt/Vt and 21.9% Vt/Vt. Trends in the same direction as those for rs10459592 were found with rs4775936, albeit less significant. The TCL1A genotype frequencies were 77.5% Wt/Wt, 20% Wt/Vt and 2.5% Vt/Vt. No association was observed between TCL1A genotypes and recurrences or MSKs, however, the small number of Vt/Vt patients on tamoxifen had greater incidence of hot flushes (HR=1.99 [0.9-3.91], p=0.09).

Conclusion: These data do not support an association between the TCL1A 3’ variant and AI-induced MSKs independent of severity. Our data do support the CYP19 SNP rs10459592 and the loosely linked rs4775936, which previously were associated with letrozole efficacy, as probably being associated with better disease outcome on anastrozole and tamoxifen and with worse drug side effects. The degree of association requires definition in further data sets.

P1-06-03 Predictive Value of HER2, Topoisomerase-II (Topo-II) and Tissue Inhibitor of Metalloproteinases (TIMP-1) for Efficacy of Taxane-Based Chemotherapy in Intermediate Risk Breast Cancer – Results from the EC-Doc Trial.

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Background:

Despite extensive research, there is still no consensus on optimal predictors for use of taxane-based chemotherapy (cht) in early breast cancer. Some studies have revealed HER2 as a significant predictive marker for efficacy of taxanes and anthracyclines. TIMP-1 and Topo-II are reported to be predictive for anthracycline efficacy. In our previous reports, both Ki-67>20% and central G3 status emerged as significant predictors for taxane benefit. We have now compared HER2 and Topo-II (as protein expression and gene amplification) and TIMP-1 immunoreactivity as well as factor combinations (HT (HER2/TIMP-1) and 2T (Topo-II/TIMP-1)) regarding their predictive value for benefit from taxane-based cht.

Methods: The EC-Doc trial randomized 1950 patients with 1-3 positive LN to 6x CEF/CMF vs. 4xE-4xD. Significantly better DFS and OS favoring EC-Doc have been previously reported (Nitz et al., SABCS 2008). Protein expression and gene amplification data as well central histology/grade were available for 772 patients. Survival analysis was performed using Cox proportional hazards and Kaplan-Meier statistics. Analysis of HER2 survival impact status was prospectively planned.

Results: The entire and the investigated study populations did not differ regarding baseline characteristics. After median follow up of 64 months, both DFS (5y 90% vs. 80%, p=0.006) and OS (5y 95% vs. 92%, p=0.022) rates significantly favored EC-Doc vs. CEF in this cohort as well. HER2 over-expression (3+ and/or FISH≥2.0) was reported in 158 tumors (20%), Topo-II aberration (deletion or amplification) was reported in 78 (49.4%) HER2+ and in 83 (13.6%) HER2-negative tumors; 496 tumors were classified as TIMP-1 immunoreactive (65.2%). None of these factors were significantly prognostic for EFS in this collective. Regarding DFS, EC-Doc was strongly superior to FEC in HER2+ tumors (HR=0.29, 95%CI: 0.12-0.7, p=0.006) but not in HER2- tumors (p=0.18). In Topo-II aberrated tumors, the benefit of EC-Doc was remarkably strong (HR=0.28, 95% CI: 0.11-0.69, p=0.006), whereas the benefit was not significant in Topo-II normal tumors (p=0.16), which comprise more than ¾ of the total. In contrast, Topo-II protein overexpression (>10%) was not associated with a stronger benefit in either subgroup. The superiority of EC-Doc to FEC was significant in the larger group of TIMP-1 immunoreactive tumors (HR=0.57, p=0.025) but not in TIMP-1 negative tumors (p=0.14), similar behavior was seen in “HT” and “2T” subgroups (significance with HR about 0.5 in the “+” subgroups).

In a multivariate model for DFS including age, tumor size, Ki-67, central grade, HR, HER2, Topo-II aberration, TIMP-1 status, therapy and interactions of all these factors with therapy arm, the only significant therapy interaction was that of (high) Ki-67 (HR=0.76, 95% CI: 0.59-0.98, p=0.03); significant main effects in this model were age, central grade, and Ki-67.

Conclusions: These data suggest predictive significance for Topo-II aberration, TIMP immunoreactivity and HER2 over-expression as well as a multivariate predictive significance of high Ki-67 for enhanced benefit of taxane-based cht.
P1-06-04
Martin M, Rodríguez-Lescure A, Stijlemans IJ, Munárriz B, Ruiz-Borrego M, Davis C, Crespo C, Rodríguez CA, Ebbert MTW, Álvarez I, Furió V, Bastien RRL, Garcia AM, Cheang MC, Palacios J, Ellis MJ, Carrasco E, Casas MI, Caballero R, Perou CM, Bernard PS. Hospital General Universitario Gregorio Marañón, Madrid, Spain; Hospital Universitario de Elche, Elche, Spain; University of Utah Health Sciences Center/Huntsman Cancer Institute, Salt Lake City, UT; Hospital Universitario La Fe, Valencia, Spain; Hospital Universitario Virgen del Rocio, Sevilla, Spain; Hospital Universitario Ramón y Cajal, Madrid, Spain; Hospital Clínico Universitario de Salamanca, Salamanca, Spain; The ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT; Hospital de Donostia, San Sebastián, Spain; Hospital Clínico Universitario San Carlos, Madrid, Spain; University of North Carolina at Chapel Hill; Washington University in St Louis, St Louis; Spanish Breast Cancer Research Group, GEICAM, San Sebastián de los Reyes, Madrid, Spain

BACKGROUND
Predictive factors of survival benefit from taxane over non-taxane based adjuvant chemotherapy regimens are needed. Recent studies have suggested that “intrinsic” breast cancer subtypes may differ in their responsiveness to specific chemotherapeutics. Tumor samples from GEICAM 9906 study (Randomized Phase 3 Trial of Fluorouracil, Epirubicin, and Cyclophosphamide Alone [FEC] or FEC Followed by Paclitaxel [FEC-P]) for Early Breast Cancer, JNCI 100:805, 2008) were gene expression profiled using the RT-qPCR PAM50 clinical test in order to identify potential predictive markers of taxane clinical benefit.

METHODS
793 formalin-fixed paraffin-embedded tumors were studied and classified into intrinsic subtypes (Luminal A & B, HER2-enriched, Basal-like, and Normal) using the PAM50 test. The assay also provided gene expression scores for the standard protein biomarkers usually measured by immunohistochemistry (ESR1/ER, PGR/PR, and ERBB2/HER2) and a meta-gene score for proliferation (proliferation signature) and for a luminal gene signature. Intrinsic subtypes, individual genes, or meta-genes were correlated with disease-free survival (DFS). Multivariable Cox regression analyses were performed to determine the significance of the interaction between treatment and intrinsic subtypes, single genes and meta-genes, adjusting for standard clinicopathological factors.

RESULTS
A 8.7 years follow-up update of GEICAM 9906 trial confirmed a statistically significant advantage of FEC-P over FEC in terms of DFS (p=0.016) and OS (p=0.013). Exploratory analyses for prognostic factors showed that treatment arm (p=0.016), tumor size (p=0.0001), type of surgery (p=0.003), tumor grade (p=0.001), nodal status (p=0.0001), intrinsic subtypes (p=0.0001), ERBB2 (p=0.031), PGR (p=0.0001), Luminal meta-gene (p=0.006) and Proliferation meta-gene (p=0.0001) were associated with DFS. A Cox multivariable analysis using the backward and forward method showed that only the treatment arm (P=0.052), tumor size (p=0.0003), nodal status (p=0.001), intrinsic subtypes (p=0.045) and Proliferation (p=0.008) were prognostic for DFS. Concerning predictive factors, exploratory analyses were performed and interaction tests were calculated. Theses analyses showed that FEC-P was superior to FEC in low PGR expression (HR= 0.68, p=0.034) and not in high PGR group (HR=0.83, p=0.245); interaction test p=0.358. Similarly, FEC-P was superior in low ERBB2 expression (HR=0.67; p=0.005) and not in the high ERBB2 group (HR=0.92, P=0.707); interaction test p=0.256. Interestingly, superiority of FEC-P was observed for the low Proliferation Signature group (HR=0.58, p=0.006) in contrast to the high proliferation group (HR=0.93, p=0.633); the interaction test showed an almost statistical significant difference (p=0.069). The FEC-P arm showed improved outcome in all genomic intrinsic subtypes, although no subtype alone reached statistical significance.

CONCLUSION
Our study suggests that the PAM50 proliferation signature could be predictive of benefit for adding weekly paclitaxel to the adjuvant chemotherapy FEC regimen. These results need further validation in an independent study.
The last two authors of this abstract have contributed equally to this study.

P1-06-05
Withdrawn by Author

P1-06-06
The Importance of CXCL10 and CXCR3-A in Breast Cancer.
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Background: CXCL10 is a chemokine with chemo attractant properties which signals through G protein-coupled receptor CXCR3. At least two reported isoforms exists; CXCR3-A and CXCR3-B. CXCL10 is involved in the recruitment of leukocytes, immune modulation and angiogenesis. In animal models CXCL10 inhibits tumour growth, metastase formation and inhibits angiogenesis. The receptors have opposite features; CXCR3-A mediates proliferative or anti-apoptotic response and CXCR3-B mediates growth inhibition and apoptosis in response to ligand binding. The aim of this study was to investigate the role of CXCL10 and CXCR3-A as prognostic or tamoxifen treatment predictive factors in breast cancer.

Material and Methods: A randomized tamoxifen trial comprising 1,780 breast cancer patients was conducted in Stockholm, Sweden, 1976-1990. All patients had lymph node negative primary breast cancer and were postmenopausal at the time of diagnosis. The patients were randomized to tamoxifen or no endocrine treatment. We analyzed the protein expression of CXCL10 and the receptor CXCR3A with immunohistochemistry using tissue microarrays, which were constructed from paraffin blocks originating from 912 patients. CXCL10 and CXCR3A could be scored in 793 (87%) and 735 (81%), respectively.

Results: High expression of CXCL10 was associated with significant benefit of tamoxifen treatment concerning local recurrence and breast cancer survival (P=0.02 and P=0.008). For patients with high CXCL10 expression, tamoxifen decreased the risk concerning local recurrence (rate ratio (RR) = 0.56, 95% C.I. 0.33-0.97, P=0.0029) and breast cancer survival (RR 0.6 95% C.I. 0.39-0.97, P=0.023). High expression of CXCR3-A was associated with significant benefit of tamoxifen treatment concerning total recurrence and breast cancer survival (P=0.00001 and P=0.004, respectively). For patients with high CXCR3-A expression, tamoxifen decreased the risk of total recurrence (RR 0.54 95% C.I. 0.35-0.83, P=0.005) and breast cancer survival (RR 0.49 95% C.I. 0.35-0.89, P=0.00003). On the opposite, in the cohort of patients with no endocrine treatment and high CXCR3-A expression, there was an increased risk of total recurrence (P=0.003).
Conclusions: This study indicates that high expression of CXCL10 can be used as predictive markers for tamoxifen treatment. Further, high expression of CXCR3-A is related to bad prognosis and predicts tamoxifen treatment in breast cancer patients. This needs to be further evaluated.

P1-06-07
TIMP-1 in Combination with HER2 and TOP2A for Prediction of Benefit from Adjuvant Anthracyclines in High Risk Breast Cancer Patients.
Hertel PB, Tu D, Ejlersen B, Jensen M-B, Balslev E, Jiang S, O’Malley FP, Pritchard Ki, Shepherd LE, Bartels A, Brünner N, Nielsen TO. Faculty of Life Sciences, Univ of Copenhagen, Copenhagen, Denmark; National Cancer Institute of Canada, Kingston, ON, Canada; Rigshospitalet, Copenhagen, Denmark; Danish Breast Cancer Cooperative Group, Copenhagen, Denmark; Herlev Hospital, Herlev, Copenhagen, Denmark; Mount Sinai Hospital, Toronto, ON, Canada; University of Toronto, Toronto, ON, Canada; BCCA - Vancouver Cancer Centre, Vancouver, BC, Canada

Purpose: HER2 amplification, TOP2A aberrations and absence of TIMP-1 (Tissue Inhibitor of Metalloproteinase-1) expression in breast carcinomas have been associated with incremental benefit from anthracycline-containing adjuvant chemotherapy in several reports. In the DBCG 89D trial, we demonstrated that the predictive value of these markers improved when they were combined in a profile and the present study was undertaken to validate these findings in NCIC CTG MA.5, a similar but independent clinical trial.

Design: TIMP-1 was examined by immunohistochemistry in archival tumor tissue from 403 of 716 premenopausal high-risk patients with known HER2 and TOP2A status who were randomized to CEF or CMF in the MA.5 trial. Patients were classified according to 2 predefined marker profiles – the HT profile (HER2,TIMP-1) and the 2T profile (TOP2A, TIMP-1) and the statistical analyses were performed as closely as possible to the analytical approach used previously in the MA.5 trial and when analysing the biomarker profiles in the DBCG 89D trial.

Results: 98 (24%) patients had no TIMP-1 staining of tumor cells, 27% were HER2 amplified, and 18% were TOP2A aberrant. 44% of patients were classified as HT responsive (HER2-positive and/or TIMP-1 negative) and 37% as 2T responsive (TOP2A aberrant and/or TIMP-1 negative). There was no heterogeneity in treatment effect of CEF versus CMF according to TIMP-1. In HT responsive patients, CEF was superior to CMF with improved RFS (adjusted HR, 0.64; 95% CI, 0.42 to 0.98) and a borderline-significant improvement in OS (adjusted HR, 0.66; 95% CI, 0.42 to 1.04). A significant HT profile versus treatment interaction was detected for OS (P=0.03). In 2T responsive patients, CEF was superior to CMF with borderline significant improvement in RFS (adjusted HR, 0.67; 95% CI, 0.43 to 1.03), and with improvement in OS (adjusted HR, 0.58; 95% CI, 0.36 to 0.93). A significant 2T profile versus treatment interaction was detected for OS (P=0.01).

Conclusion: In the MA.5 trial, we have validated the HT and 2T profiles as predictors of incremental benefit from anthracycline-containing chemotherapy. The proportion of patients categorized as anthracycline responsive increases from 18-27% when using individual markers to 37-44% when combining TIMP-1 with either HER2 or TOP2A. Patients with responsive profiles had a 34-42% relative reduction in mortality when treated with CEF. In contrast, patients with non-responsive profiles (56-63% of patients) had no incremental benefit from CEF compared with CMF. All 3 biomarkers are easily applied in the pathology lab and as such could be used in daily clinical practice to select patients for anthracycline or non-anthracycline containing adjuvant chemotherapy.

P1-06-08
Effect of Treatment Emergent Symptoms on Relapse Free Survival: NCIC CTG MA.12 a Randomized Placebo-Controlled Trial of Tamoxifen after Adjuvant Chemotherapy in Pre-Menopausal Women in Early Breast Cancer.
Chapman J-AW, Shepherd LE, Le Maitre A, Pritchard Ki, Graham BC, Gelmon KA, Bramwell VH. NCIC Clinical Trials Group, Queen’s University, Kingston, ON, Canada; University of Toronto, Toronto, ON, Canada; BCCA - Vancouver Cancer Centre, Vancouver, BC, Canada; Tom Baker Cancer Centre, Calgary, AB, Canada

Background: Evidence from the ATAC trial that vasomotor or joint symptomatology by 3 months is associated with reduced recurrence after 3 months led to an interest in examining this phenomenon in other aromatase inhibitor trials. We examined here whether there is such an association in the context of a placebo-controlled tamoxifen therapy trial.

Methods: NCIC CTG MA.12 is a randomized placebo-controlled trial of tamoxifen (TAM) after adjuvant chemotherapy for pre-menopausal women with early breast cancer. Eligible patients were included if they received some protocol therapy, were alive and disease-free at 3 months: 1.) without prior grade 3/4 vasomotor or joint symptoms (N=293; only 3 patients had prior grade 1/2 vasomotor or joint symptoms); separately, 2.) all patients with/without prior vasomotor or joint symptoms (N=631). Vasomotor symptom at 3 months was adverse reporting of any grade of hot flashes and/or sweating, while joint symptom was any adverse event reporting of pain – joint, pain – muscle, pain – bone, arthritis, joint – function, or musculoskeletal – other. Exact Fisher tests were used to examine associations between baseline patient and tumour characteristics, treatment arm, and the development of symptomatology. Univariate testing of effect of symptomatology on relapse-free survival (RFS) was with a stratified Cox model, and multivariate was with stratified step-wise forward Cox modeling.

Results: MA.12 accrued 672 patients, and the median follow-up for this investigation was 9.7 years. Excluding patients with prior vasomotor or joint symptoms, 27.3% of 293 patients reported vasomotor or joint symptoms by three months, all of which was vasomotor. Meanwhile, 20.8% of all 631 patients had symptomatology by 3 months: 19.2% reported vasomotor alone, 1.1% joint alone, and 0.5% both. With no prior symptoms, 23.4% on placebo (P) and 31.7% on TAM developed symptomatology; age was the only baseline factor with significant differences at 3 months (p=0.01), with under 40 years 18% of women on TAM and 8% on P, and 50 or older, 21% on TAM and 14% on P being symptomatic. For all patients, 20.1% on P and 21.4% on TAM reported symptomatology by 3 months, and there was weak evidence that those >50 on TAM had more symptoms (p=0.06). Vasomotor and joint symptoms did not exhibit significant univariate or multivariate effects on RFS (without prior symptoms, respectively, p=0.98 and p=0.90; for all patients, p=0.99 and p=0.93).

Discussion: We did not observe any association of vasomotor or joint symptoms by 3 months and relapse-free survival after 3 months in the MA.12 placebo-controlled trial of tamoxifen therapy in pre-menopausal women. This mirrors the results we observed in our MA.27 trial of exemestane versus anastrozole in postmenopausal women, and points to the simple metric of early symptomatology not being a universal predictor of reduced relapse.
P1-06-09
Patient-Specific Integrative Pathway Analysis Using PARADIGM Identifies Key Activities in I-SPY 1 Breast Cancer Patients (CALGB 150007/150012; ACRIN 6657).
Wolf DM, You C, Benz S, Vaske C, Stuart J, Roy R, Oshen A, Boudreau A, Haussler D, Gray J, Spellman P, Davis S, Hylton N, Van ‘t Veer L, Esserman L. University of California, San Francisco; University of California, Santa Cruz; Oregon Health & Science University; I-SPY 1 Trial Investigators

Background: A major challenge in interpreting high-throughput multianalyte genomic data sets such as those produced by the ISPY clinical trials is data integration and interpretation within the context of biologically relevant pathways. To address this need, the data analysis tool PARADIGM (Pathway Recognition Algorithm using Data Integration on Genomic Models) was developed to infer the activities of genetic pathways by integrating any number of functional genomic data sets for a given patient sample into a pathway activity profile.

Methods: We used PARADIGM to integrate gene expression (Agilent 44K) and DNA copy number data (AFFY 22K and 330K MIP) from 133 ISPY-1 patients into pathway component activity levels for approximately 1400 curated signal transduction, transcriptional and metabolic pathways superimposed onto a single non-redundant “SuperPathway”. These pathway activities then become the substrate for statistical analyses to identify pathways characterizing different breast cancer subtypes, as well as those associated with recurrence and response to neoadjuvant chemotherapy within breast cancer subgroups. To identify subtype-specific pathway activities, we used ANOVA for initial feature filtering followed by Tukey analysis with Benjamini Hochberg multiple testing correction. For other binary outcome comparisons we used Mann-Whitney (2-sample Wilcoxon) analysis. PARADIGM results were corroborated with pathway enrichment analysis and filtered for significance.

Results: In agreement with breast cancer cell line and other prior studies, basal-like and triple negative cancers are dominated by upregulation of the FOXM1 and MYC/Max subnetworks and downregulation of the FOXA1/ER signal transduction pathway, the converse of the activity pattern seen in luminal breast cancers. These and other subtype associations pass stringent multiple testing corrected significance tests. Though an association study of recurrence over the entire patient cohort mostly yields pathways characteristic of basal-like tumors, alternative pathway associations emerge when subtypes are analyzed individually for outcome and significance tests are relaxed to include features that pass un-corrected Wilcoxon significance tests and also generate highly significant pathway enrichment scores. Subtype-specific drivers of recurrence and chemoresistance supported by this level of evidence include ALK1/2 (TGFβ-BMP) and p53 effector signaling for basal and Syndecan-1 and c-MYC for luminals. Chemo-sensitivity pathways, assessed by association with pCR and RCB1, appear to be subtype-specific as well, with HDAC class 1 signaling, LRP6-Wnt, and IRE1α chaperones dominating basal-like cancers and c-MYB activity dominating Her2+ cancers, whereas chemo-sensitivity of HR+/Her2- cancers though rare appears to be driven by the DNA damage axis (BRCA1/BARD1).

Conclusion: These and other similar analyses suggest that patients with TN or basal-like disease might benefit from the addition of ALK1 pathway inhibitors to treatment, whereas high risk HR+ patients might benefit from Syndecan-1 inhibitors. C-MYC/MAX inhibitors might benefit all high risk patients.

P1-06-10
Lobular Breast Cancer and NAC: Combined Results from the NKI and I-SPY 1 Trial.
Mukhtar RA, Lips E, Wesseling J, Livasy C, Yau C, Berry D, van’t Veer L, Carey LA, Esserman LJ, Rodenhuis S, I-SPY Investigators, Hwang ES. UCSF; Netherlands Cancer Institute; University of North Carolina, Chapel Hill; MD Anderson

Background
The benefits of neoadjuvant chemotherapy (NAC) in breast cancer are twofold: allowing breast conservation surgery, and assessment of response. Having pathologic complete response (pCR) predicts improved outcomes. Many have suggested that invasive lobular cancers (ILC) do not respond to NAC as well as invasive ductal cancers (IDC), and recommend against offering NAC in such cases. We previously investigated differences in the response to NAC in ILC and IDC in the I-SPY 1 Trial, and now present findings from a joint analysis with the Netherlands Cancer Institute (NKI).

Methods
We combined datasets comprising 676 patients (221 from I-SPY, 455 from NKI) enrolled in NAC trials. Eligible patients had palpable tumors ≥ 3 cm, and underwent serial biopsies, microarray analysis (Agilent 44K for I-SPY and Illumina 6v3 for NKI), and MRI imaging. ILC versus IDC histology was assigned by pathologic appearance, and ILCs were tested for e-cadherin expression and centrally reviewed by a breast pathologist at each site. We performed multivariate logistic regression analyses to assess differences in pCR. We compared intrinsic subtypes, prognostic gene signature expression, and MRI phenotypes.

Results
There were 75 ILC and 601 IDC cases. ILCs had lower risk features and were more likely hormone receptor + (Table 1) but there was substantial heterogeneity and many ILC had high risk features. There were fewer T2 (33% versus 48%) but more T3 tumors (59% versus 37%) among the ILCs. Nearly 10% of ILC cases had the basal intrinsic subtype; 22% expressed the activated wound healing signature, and 56% had a high risk 70-gene prognostic signature. While pCR rate was lower in ILCs, adjustment for low risk features showed lobular histology was not an independent predictor of pCR. ILC was less likely to present as a solid mass. 85% of ILC were e-cadherin negative.

Characteristics of ILC versus IDC

<table>
<thead>
<tr>
<th>PathR</th>
<th>Overall</th>
<th>ILC</th>
<th>IDC</th>
<th>pvalue</th>
</tr>
</thead>
<tbody>
<tr>
<td>pCR</td>
<td>352 (22.7%)</td>
<td>10 (10.7%)</td>
<td>242 (24.2%)</td>
<td>0.008</td>
</tr>
<tr>
<td>Grade 3</td>
<td>214 (43.9%)</td>
<td>3 (1.9%)</td>
<td>211 (42.6%)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Marker subtypes

| HR+/Her2- | 331 (49.3%) | 59 (78.7%) | 272 (45.6%) |
| HR+/Her2+ | 92 (13.7%) | 10 (13.3%) | 82 (13.3%) |
| HR-/Her2- | 213 (32.6%) | 3 (4%) | 210 (35.4%) |
| HR-/Her2+ | 66 (14.7%) | 3 (4%) | 63 (12.6%) |

Intrinsic Subtypes

| Lum A | 131 (31.6%) | 29 (70.7%) | 102 (27.4%) |
| Lum B | 82 (19.5%) | 2 (4.9%) | 80 (21.5%) |
| Basal | 86 (15.9%) | 2 (4.9%) | 84 (22.7%) |
| Normal | 34 (8.2%) | 4 (9.8%) | 30 (8%) |

Wound healing sig activated | 250 (60.4%) | 9 (22%) | 241 (64.6%) | <0.001 |

High risk 70 gene sig | 359 (86.7%) | 25 (56%) | 334 (90%) | <0.001 |

MRI Pattern

| Mass | 379 (41.5%) | 6 (13%) | 373 (44.9%) |
| Multinodular | 127 (29.5%) | 26 (56.5%) | 101 (26.5%) |
| P1-06-10 Diffuse 125 (29%) 14 (30.4%) 111 (28.8%) |

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Conclusions

This combined analysis suggests that the decreased response to NAC in ILC is due to the association between ILC and low risk features. ILC in a clinically high risk setting has a higher proportion of high risk biology in spite of lobular histology. Lobular histology alone should not be used to exclude patients from NAC trials or treatment.

P1-06-11

Comparison of Community and Central Her2 Assessment on Outcome of Neoadjuvant Chemotherapy in the I-SPY Trial.

DeMichele A, Vau C, Zhu J, Wuhufkuhle J, Lenburg M, Buxton M, Davis S, Mies C, Livasy C, Chin K, Gray J, Carey L, Esserman L, Petricoin E. Abramson Cancer Center, University of Pennsylvania, Philadelphia, PA; University of California, San Francisco, CA; George Mason University, Fairfax, VA; University of California, Santa Cruz, CA; Carolina HealthCare System, Charlotte, NC; Oregon Health Sciences University, Portland, OR; The University of North Carolina at Chapel Hill, Chapel Hill, NC; University of Pennsylvania, Philadelphia, PA

Background: Her-2/neu overexpression, by immunohistochemistry (IHC) or fluorescence in-situ hybridization (FISH), is highly correlated with response to trastuzumab and these are currently the gold-standard, FDA-cleared testing methods for assigning treatment to Her-2-directed therapies. However, substantial variability has been documented between community and central laboratory IHC and FISH testing. Biologically, Her-2 overexpression may reflect increased gene copy number, gene expression and/or protein production, and these can be measured by other platforms, including comparative genomic hybridization (CGH), expression arrays and quantitative protein assays, respectively. We sought to determine the degree to which community IHC/FISH results differed from centrally-assessed IHC, FISH, and other assessment platforms within the I-SPY Trial and whether response to neoadjuvant chemotherapy (NAC) differed by platform.

Methods: The I-SPY Trial enrolled 237 women 2002-06 with invasive breast tumors at least 3 cm in clinical/radiographic size who subsequently underwent anthracycline/taxane NAC, serial core biopsies and imaging. Pathologic complete response (pCR) was determined at time of surgery and 3-year follow up has been reached. Trastuzumab was given to Her2+ patients at physician discretion, based upon community IHC/FISH results, and became more widespread after 2005. Central I-SPY laboratories determined Her2 copy number by MIP array, gene expression by Affymetrix and Agilent arrays, and Her2 protein by reverse-phase protein array (RPMA). Unsupervised clustering algorithms were used to evaluate expression patterns. Composite variables were constructed for DNA, RNA and protein positivity as well as for community and central IHC/FISH. Platforms were compared and Kaplan-Meier curves were constructed to compare outcomes by platform.

Results: 222 women were evaluable, though not all patients had results for all platforms. Community composite IHC/FISH was positive in 64/214 (30%) but only 41 of these (64%) were confirmed by central IHC/FISH and 4 additional cases were centrally positive despite negative community testing. Concordance was high among centrally-assessed Her2 platforms, but was lower between community IHC/protein and central RNA (90%), DNA (91%) and protein (91%). Among patients receiving trastuzumab (n=36), the pCR rate was ~50% regardless of Her2-assessment platform; in contrast, those not receiving trastuzumab had pCR rates below 30%. Among the 64 patients deemed Her2+ by community IHC/FISH, 30 (48%) had pCR and 15 (25%) have had distant relapse. Five distant relapses have occurred despite pCR; all received trastuzumab, all were Her2 positive by multiple central platforms and 3/5 were ER-positive. Sites of distant relapse included brain, bone and viscera; only 1 of 5 had isolated brain relapse.

Conclusions: Community IHC/FISH testing for Her2 expression in the I-SPY Trial overcalled Her2 positivity compared to central testing while central results were highly concordant among DNA, RNA and protein platforms. Despite the high rate of community “false positives”, relapse after pCR occurred only in central Her2 “true positives,” exclusively among those receiving trastuzumab, and was rarely isolated to CNS sanctuary sites.

P1-06-12

Circulating Tumor Cells (CTC) Monitoring during Phase II Study with Lapatinib (L) and Capecitabine (C) in Patients with Brain Metastases from HER2-Positive (+) Metastatic Breast Cancer (MBC) before Whole Brain Radiotherapy (WBR): LANDSCAPE Study.

Pierga J-Y, Cropet C, Tresca P, Dalenc F, Romieu G, Campone M, Mahier Ait-Oukhatar C, Le Rhun E, Gonçalves A, Lebeurteur M, Domont J, Gutierrez M, Curé H, Ferrero J-M, Labbe-Devilliers C, Bidard F-C, Bachelot T, Institut Curie & Universite Paris Descartes, Paris, France; Centre Léon Bérard, Lyon, France; Institut Claudius Regaud, Toulouse, France; Centre Val d’Aurelle, Montpellier, France; Institut de Cancérologie de l’Ouest, Nantes, France; R&D Unicancer; Paris, France; Centre Oscar Lambret, Lille, France; Institut Paoli Calmettes, Marseille, France; Centre Henri Becquerel, Rouen, France; Institut Gustave Roussy, Villejuif, France; Institut Curie - Hôpital René Huguenin, Saint-Cloud, France; Institut Jean Godinot, Reims, France; Centre Antoine Lacassagne, Nice, France

Background: Decrease of CTC level during treatment in MBC has been reported as an independent prognostic and predictive factor of patients’ outcome. Monitoring CTC in addition to clinical response criteria is currently evaluated in early clinical trials in various cancer types. We sought to evaluate the clinical interest of peripheral blood CTC for patients included in the LANDSCAPE study which assessed the efficacy of upfront systemic treatment with L+C for newly diagnosed brain metastasis.

Methods: This analysis is a preplanned secondary endpoint of the LANDSCAPE study. Eligible pts had HER2+ MBC with BM not previously treated with WBR, C or L. Pts received L1250 mg/day and C2000 mg/m²/day, days 1-14, every 21 days. The primary endpoint was a centrally assessed CNS objective response (CNS-OR) defined as a ≥50% volumetric reduction of CNS lesions in the absence of increasing steroid use, progressive neurologic symptoms or progressive extra-CNS disease. CTC were detected in 7.5 ml of blood using the CellSearchSystem™, combining EpCAM immunomagnetic selection (IMS) followed by anti-cytokeratin (A45B/B3) fluorescently staining for CTC at baseline and at day (D) 21, before cycle 2.

Results: From 04/2009 to 08/2010, 45 pts were enrolled, 41 were evaluable for CTC at baseline and 38 at D21. Median age was 56 (range 35 to 79). PS was >1 only in 2 pts. At baseline, 20/41 (48.8%) pts had ≥1 CTC and 9 (22%) ≥5 CTC (range 1-301, median 3). CTC were detected in pts with (18/37) or without disease outside SNC (2/6) (p=0.63). At a median follow-up of 10 months (range 2.9-16.5), median TTP was 6.0 months [95% CI 4.9; 7.4] vs. 4.3 [2.8; 5.9] months for pts without and with CTC at baseline respectively (p=0.14). After 21 days of treatment, a disappearance of CTC was observed in 11pts (31%). At D21, only 7 (18.4%) pts had ≥1 CTC and 3 (8%) ≥ 5 CTC (p=0.006, D21 vs. baseline). In 43 evaluable pts, CNS-OR rate was 67% (95%CI 51-81), with a median time
from inclusion to response of 1.8 month. Absence of CTC was not correlated with CNS-OR rate at baseline (17/21 (81%) vs. 11/19 (58%), NS). Strikingly, remaining positivity for CTC at D 21 (≥ 1CTC) was correlated with a poor response rate in CNS: 2/6 (33.3%) vs. 25/31 (80.6%) in pts with 0 CTC, p=0.03.

**Conclusions:** Early decrease (at D 21) in CTC level is correlated with a high response rate in newly diagnosed BM to L + C and underlines the predictive value of this blood marker in MBC pts even for brain metastasis. Longer follow-up is needed to assess its prognostic value under antiHER2 targeted therapy.

### P1-06-13

**An Amplicon-Driven Aromatase Inhibitor Response (ADAIR) Signature Provides an Orthogonal Risk Classifier for ER+ Breast Cancer.**


**Background:** Many gene signatures have been proposed to predict outcomes for estrogen receptor positive (ER+) breast cancer; most are solely based on mRNA expression data without integration of genomic aberrations that are the primary drivers of disease. We coupled gene expression and copy number variation data to improve the current generation of prognostic algorithms.

**Methods:** mRNA expression based discovery was conducted in 167 patients with low (<10%) or high (>10%) levels of Ki67 after neoadjuvant aromatase inhibition. Genes that were significant in SAM analysis (q ≤0.05) were used if significantly correlated (P≤0.05) with copy number gains detected by aCGH. Interrogation for association with relapse-free survival (RFS) (P≤0.05) in a large public microarray dataset produced an Amplicon-Driven Aromatase Inhibitor Response (ADAIR) signature. Each gene was subject to rigorous independent validation in public microarray datasets and by NanoString on archival tumor RNA accrued from patients treated with adjuvant tamoxifen (UBC-TAM) that were previously profiled for PAM50 subtyping and risk of relapse (RO). The associations between clinicopathological outcomes and management of breast cancer (BC).

**Results:** A 54-gene ADAIR signature of 27 FR(favorable response) and 27 UR(unfavorable response) genes was chosen based on statistical and genomic information. 80% of the ADAIR genes were univariately prognostic for RFS in UBC-TAM. The multigene-based ADAIR risk classifier of endocrine sensitive, intermediate and insensitive categories were prognostic for relapse in the combined public data (p=2.72e-08) and UBC-TAM (p=1.51e-08). Multivariable survival analysis showed that ADAIR was independently prognostic from standard clinical variables. The ADAIR risk classifier was highly concordant with the PAM50-gene based intrinsic subtype and ROR using subtype information (ROR-S) in all datasets in the analysis (for ROR-S, p=3.50e-44 in combined public data and p=2.16e-47 in UBC-TAM). ADAIR significantly stratified the patients in the medium ROR subtype risk group (p=0.007 in public cohort, p=0.005 in TAM), suggesting clinical utility. Pathway analysis indicated that the FR gene signature was enriched for cell survival genes, while UR genes were largely cell cycle related. Two major TFs, E2F1 and GABPB1 were predicted to regulate 22 and 13 signature genes. Amplification/overexpression of E2F1 regulated genes characterizes the UR signature. In contrast, the FR signature down regulates the transcriptional repressor GABPB1, resulting in upregulated NFKB1 possibly mediating a survival response.

**Conclusions:** These data suggest that current gene expression signatures can be improved upon through the inclusion of genes whose over-expression is linked to the gene copy number gains typical of the ER+ breast cancer genome. Functionally, tumors sensitive to estrogen deprivation are associated with genes that promote cell survival, whereas resistant tumors are associated with genes that drive estrogen independent cell cycle progression. This study underscores the profound differences in the transcriptome of estrogen-dependent and independent breast cancer beyond the patterns identified by the established classifiers.

### P1-06-14

**Topoisomerase II alpha (Top2a) Protein Expression Is a Predictor for Response to Anthracycline-Based Chemotherapy (ATC-CT): Is It Due to Gene Amplification, HER2-Coamplification or a Summation of Pathways Leading to This Highly Proliferative Phenotype?**

Abdel-Fatah TMA, Lambros MB, Vatcheva R, Ball G, Dickinson PD, Moseley P, Green AR, Ellis IO, Reis-Filho JS, Chan S. Nottingham University City Hospital, NHS Trust, Nottingham, Nottinghamshire, United Kingdom; The Institute of Cancer Research, London, United Kingdom; School of Molecular Medical Science, Nottingham University, Nottingham, Nottingham Trent University, Nottingham, United Kingdom

**Background:** The evaluation of Top2a protein may be clinically more useful than gene alterations as a predictive marker for ATC-CT. In this study we assessed the association between gene copy number, gene and protein expressions of both TOP2A and HER2, and their effect on clinicopathological outcomes and management of breast cancer (BC).

**Methods:** 1- To study the response to anthracycline based chemotherapy (ATC-CT): The associations between clinical outcomes and both gene copy number changes (using in-situ hybridization; CISH) and protein expression (using immunohistochemistry) were studied in the neoadjuvant and adjuvant settings: a) 250 locally advanced primary BC treated with Neoadjuvant ATC-CT with or without Taxane followed by surgery (S) + radiotherapy (RT); pathological complete response (pCR) was used as the primary end point (PEP), b) 245 BC in which all patients were treated with S + RT followed by Adjuvant ATC-CT; progression free survival (PFS) was used as PEP (i) 145 primary BC overexpressing HER-2 treated with S+ RT followed by sequential adjuvant ATC-CT+ trastuzumab; PFS was used as PEP.

2- To study the clinic-pathological association of TOP2A alterations, we evaluated TOP2A alterations detected by CISH and IHC in an unselected series of 1650 consecutive cases of primary BC who treated with S + RT and received adjuvant CMF and/or endocrine therapies according to Nottingham prognostic index and ER status. 3- To study in details the molecular alterations of TOP2A/HER2, in 171 unselected series of primary BC, we evaluated a) gene copy number changes using both high resolution oligo array CGH and CISH, b) mRNA expression using Agilent gene expression array and c) protein expression using IHC. We analysed 48,000 gene transcripts using Artificial Neural Networks (ANN) and pathway analysis to identify genes and biological pathways that related to TOP2A gene alterations.

**Results:**

1) In the ATC-CT neoadjuvant series, the pCR rate was 32/115 (28%) in tumours expressing high levels of Topo2A, compared to 5/74 (7%) in tumours expressing low levels of Topo2A (p=0.0001).
In multivariate analysis, Top2A overexpression was an independent predictor for pCR (HR 5.1, CI 95%; 1.4-18.4, p<0.001).

2) Top2A overexpression was strongly associated with mitotic index, histological grade, KIF2C, loss of p53 function and the absence of both BRCA1 and ATM inactivation (p<0.0001).

3) ANN and pathway analysis revealed that Top2A-strongly correlated genes are involved in: mitotic cell cycle regulation especially M phase and cell division (AURKB, KIF2C, BRIC5, ASPM, CCNA2, BUB1, FBX05, PTG1, CDC5, CDC3, CDC8), Kinesin and microtubules regulator genes (KIF2C, KIF11, KIF14, KIF20A, KIF23, and KIFC1), and metastases (BRIC5, BUB1B, CCNA2, CCNE, PRGG1, PRM2, STMN1).

Conclusions: Top2A protein expression is an independent predictor for pCR after ACT-CT treatment. The high response rate of Top2A protein overexpression supports the theory that Top2a protein is a direct target of ATC-CT in these highly proliferative tumour cells. Furthermore, evaluation of Top2A protein may lead to a clinically useful test.

P1-06-15
A Genomic Predictor Developed from Breast Cancer Cell Lines Predicts Both Disease-Free Survival and Overall Survival in Breast Cancer Patients Treated with Doxorubicin and Cyclophosphamide: A Collaborative Project of the NSABP and Precision Therapeutics.


Background: A cell line-derived multigene predictor of tumor response to doxorubicin + cyclophosphamide (MGP-AC) has been shown to predict the pathological complete response (pCR) in breast cancer patients from the National Surgical Adjuvant Breast and Bowel Project (NSABP) Protocol B-27. However, a cell line-derived MPG for doxorubicin + cyclophosphamide + docetaxel (MGP-ACT) was not predictive in patients from B-27. The purpose of this study was to further assess the performance of these predictors on disease-free survival (DFS) and overall survival (OS) in the same patient populations.

Methods: NSABP B-27 was a 3-arm trial of 2411 early-stage breast cancer patients randomized to receive 4 cycles of preoperative doxorubicin+cyclophosphamide (AC) or 4 cycles of AC followed by 4 cycles of docetaxel either pre-op (AC+T) or post-op (AC→T). MGPs for AC and ACT were developed based on the in vitro assay and microarray genomic profiles of 40 breast cancer cell lines. A higher MGP score indicates lower chemoresponse sensitivity.

Results: 322 patients with available microarray data were included for this analysis (103 treated with AC, 102 with AC+T, and 117 with AC→T). For patients treated with AC, a higher MGP-AC score was significantly associated with increased risk of disease progression (standardized hazard ratio [HR] SD set to 1=1.48, 95% confidence interval [CI]=1.02-2.15, p=0.043) or death (standardized HR=1.66, 95% CI=1.06-2.62, p=0.028) after adjusting for clinical covariates (ER status, clinical tumor size, lymph node status, and age). The addition of MGP-AC to the clinical model improved the accuracy in predicting five-year DFS: the area under the ROC curve improved from 63% to 72%. For patients treated with AC+T or AC→T, MGP-ACT was not predictive of either DFS (standardized HR=1.03, 95% CI=0.78-1.37, p=0.818) or OS (standardized HR=1.05, 95% CI=0.73-1.51, p=0.8).

Conclusions: A cell line-derived MGP for AC that was predictive of pCR was also predictive of DFS and OS in breast cancer patients treated with neoadjuvant AC. The MGP for ACT, which was not predictive for pCR, was not predictive of either DFS or OS in patients who received docetaxel after AC. The B-27 study was funded by NCI PHS grants U10-CA-37377, U10-CA-69974, U10-CA-12027, U10-CA-69651, and U24-CA-114732, and received additional support from sanofi-aventis.

P1-06-16
BRCA2 Mutation Carriers Respond Poorly to Conventional Anthracyclins/Taxanes-Based Neo-Adjuvant Chemotherapy.

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Background: BRCA2 carriers typically develop luminal B-type breast cancers. Their exact sensitivity to conventional chemotherapy remains unclear. Promising drugs such as parp inhibitors are currently being developed in this indication. We attempted to evaluate pathological response rates of BRCA2 carriers in current the routine setting.

Patients and methods: We screened our database of all patients seen at our genetic clinic over the past 15 years who were completely tested for BRCA1 and BRCA2 mutation and had received primary anthracyclines/taxanes-based chemotherapy (6-8 cycles) for invasive breast cancer as primary care. The pathological responses of pts who tested positive for BRCA2 were compared to those of BRCA1 and BRCA2-negative patients who had been diagnosed with luminal breast cancer (ER-positive, Her2-negative).

Results: Among 155 BRCA2-carriers and 503 wild-type patients, 24 BRCA2 pts and 58 pts with luminal B-type breast cancer have received primary standard anthracyclin/taxane-based chemotherapy. Median age was 38 for both populations. Median tumor size was 45 and 40 mm respectively among carriers and WT pts. 60% of BRCA2 and 100% of WT luminal B pts had ER-positive disease. A pathological complete response occurred in 18% of BRCA2 carriers and of 39% of luminal B Wt-pts. 65% of BRCA2 and 51% of WT-pts pts remained node-negative.

Conclusion: Deleterious germline mutations of BRCA2 are associated with a low probability of complete pathological response and a high risk of axillary invasion after conventional primary chemotherapy. Alternative treatments are highly expected.

P1-06-17
A New Pathological Response Index (PRI) for Neoadjuvant Chemotherapy Accurately Predicts Clinical Outcomes of Locally Advanced Breast Cancers (LAPBC).

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Pathological complete response (pCR) after neoadjuvant chemotherapy predicts overall survival that is independent of treatment regimen. However, pCR is not a perfect surrogate for overall survival, given that a significant number of patients who did not achieve pCR also have a good prognosis and a small number of patients with pCR still develop recurrences. Furthermore, residual cancer cells after neoadjuvant therapy includes a wide range of responses from near pCR to complete resistance.

In this study we performed a comprehensive pathological assessment
for 245 surgical specimens of LAPBC, treated at a single institution, removed after receiving neoadjuvant anthracycline combination chemotherapy only (n=152) or with Taxane (n=93) with median follow up 42 months. Progression free survival (PFS) was used as the study endpoint. For comparison, residual cancer burden (RCB) was calculated using web calculator at www.mdanderson.org/breast_BCR. A multivariate Cox regression model revealed that size of the residual invasive carcinoma (OR; 3.2, CI 95%; 1.6-6.1, p=0.001), presence of lympho-vascular invasion (OR; 2.4, CI 95%; 1.3-4.3, p=0.004), absence of any pathological evidence of response at the site of the primary tumour or axillary lymph nodes after CT (OR; 3.2, CI 95%; 2.2-8.0, p=0.00001) and presence of at least one apical lymph node metastases (OR; 4.2, CI 95%; 1.5-6.8, p=0.003) at surgery were significantly associated with shorter PFS. These results were used to develop a pathological response index (PRI) from which 5 prognostic subgroups with distinct clinical outcomes were identified.

Patients with PRI-PG1 (n=110; 49%) had a good clinical outcomes in both ER+ (3-year PFS; 91%) and ER- tumours (3-year PFS; 84%). Moreover, patients with PRI-PG1 who did not show pCR (n=69) had equivalent overall and PFS as those with PRI-PG1 who achieved pCR (n=41); p=NS. Patients with PRI-PG2-5, had a 5 fold increase in the risk of progression compared to those with PRI-PG1 (HR; 5.2, CI 95%; 2.9-9.6, p=0.0001). ER+ patients with PRI-PG3-5 (25% of ER+ cases) had shorter 3-year PFS 30%) compared to those ER+ with PRI PG1-2 (91%) despite treatment with adjuvant hormonal therapy (HR=6.5, CI 95%; 2.8-14.7, p=0.0001). Similarly, HER2 positive patients with PRI- PG3-5 (20% of HER2+ patients) showed very rapid progression within < 3 years (PFS; 10%) vs. those with PRI-PG1-2 (PFS; 82%) despite trastuzumab treatment (HR=8.4; CI 95%; 2.8-25.0, p=0.0001).

In conclusion, a pathological response index including size of residual tumour, post chemotherapy lymph node pathological stage, lympho-vascular invasion and any evidence of fibrotic/response reaction following neoadjuvant chemotherapy may accurately predict the chance of disease progression, identify a greater proportion of patients (>twice as many as pCR) who could potentially benefit from the neoadjuvant chemotherapy and may be able to spared further adjuvant therapy. Furthermore this new PRI may help to improve the sensitivity of pathological response as a study end-point for predicting tumours response to a given neoadjuvant regimen and enable biological markers to be studied in a good prognostic group in addition to pCR.

P1-06-18
Loss of E-Cadherin Expression in Clinical Breast Cancer Is Associated with an Adverse Outcome on Tamoxifen.
Hiscox S, Rakha E, Smith C, Farrow L, Gandahara S, Green A, Ellis I, Barrett-Lee P, Nicholson R, Geer J, Cardiff University, Cardiff, Wales, United Kingdom; Nottingham University Hospital, Nottingham, United Kingdom; Velindre Hospital, Cardiff, Wales, United Kingdom

Background: Despite the benefits provided by tamoxifen, not all ER+ patients respond to endocrine therapy and a substantial number of patients who initially respond ultimately relapse whilst on therapy, with endocrine resistance associated with poorer survival. The ability to identify biomarkers predictive of outcome on endocrine treatment would enable more effective stratification of patients most likely to benefit from such measures. Previously, we reported that tamoxifen is able to promote the development of a highly aggressive phenotype in ER+, HER2- endocrine-sensitive breast cancer cells in vitro that have been depleted of the intercellular adhesion molecule, E-cadherin. Here we have investigated the clinical relevance of these observations by examining the association between E-cadherin protein expression and clinical outcome in a series of primary early stage (Stage I-IIIA) invasive ductal carcinomas of the breast.

Material and Methods: A series of 794 ER+ breast cancer tissues of known E-cadherin immunohistochemical status (Rakha EA et al (2005) Histopathol 46(6)) were available for this study and comprised both patients who had received tamoxifen for 5 years (n=345) or who had had no endocrine treatment (n=449). E-cadherin was further assessed in a group of ER-negative, tamoxifen-treated primary breast cancers (n=93) as an internal control. The median E-cadherin H-score staining was 120 (range 0 to 300).

Results: Kaplan-Meier survival analysis revealed a significant association between reduced E-cadherin expression (< median) and overall survival (OS) in the tamoxifen-treated group at 20 years (p=0.04; Hazard Ratio [HR]=1.51, 95% CI:1.01-2.26) however this relationship was much stronger at 5 years (p=0.02; HR=2.00, 95% CI:1.10-3.65). In the ER+, tamoxifen-untreated tumours, a relationship was observed at 60 months only. Reduced E-cadherin also correlated with metastasis (regional and distant, p=0.01; HR=1.90, 95%CI:1.12-3.24) but only within the tamoxifen-treated group and only within the first 5 years, reflecting the time during which patients would have received tamoxifen. In the ER+, tamoxifen-treated cohort, no adverse impact of reduced E cadherin on these parameters was observed. Subgroup analysis of tamoxifen-treated patients revealed that the association between E-cadherin loss and adverse outcome was apparent in both HER2+ (n=39) and HER2- (n=302) cohorts. However, the association was more significant within the HER2+ group (p=0.02 for OS; HR=3.45, 95% CI:1.08-11.07) (p=0.03 for regional and distant metastasis; HR=2.78, 95%CI:1.02-7.57). These data support our in vitro observations that suppression of E-cadherin within in ER+ HER2+ breast cancer cell lines results in a significant gain in migration and invasion in response to tamoxifen.

Discussion: These results are supportive of our hypothesis that low or absent E-cadherin expression in ER+, ductal breast cancer treated with tamoxifen predicts poor survival, particularly within the ER+, HER2+ phenotype. Of note, the presence of tamoxifen appears to be required for this event, suggesting that there is a patient cohort where other systemic endocrine therapy should be considered. E-cadherin represents a biomarker able to discriminate such cases.

P1-06-19
Absence or Low Levels of c-JunNH2- Terminal Protein Kinase (JNK) Mitogen Activated Protein Kinase (MAPK) Is Related to a Luminal B Subtype and an Impaired Survival in Patients with an ER Positive Breast Cancer Treated with Adjuvant Tamoxifen.
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Background: Resistance to endocrine therapy is a clinical problem and is not explained by lack or loss of the oestrogen receptor (ER). Up-regulation of several receptor tyrosine kinases have been correlated to diminished effect of endocrine treatment. More contradictory are the role of downstream mitogen activated protein kinases (MAPK’s).

Patients and Methods: We performed a tissue micro array (TMA) on patients that originate from an earlier presented population of 404 patients with a steroid receptor positive BC subjected to tamoxifen as only adjuvant systemic treatment. Material from 120 patients was available for the TMA. JNK, p38 and ERK was determined by use of immunohistochemistry and scored 0, +1, +2, +3. Patients were
grouped into two categories for statistical analyses. Results: Absence or low JNK was statistically significantly correlated with HER2+ (p = 0.018), a Luminal B like subtype (ER+, high proliferation rate and/or HER2+) (p = 0.007), shorter relapse-free (p = 0.0494) and breast cancer corrected survival (p = 0.0458). High ERK was more frequent among no node-negative patients (p = 0.00469) and more frequent in relapse-free patients; RFS (p = 0.225); BCCS (p = 0.0705). High expression of p38 was associated with p38 previously determined by ELISA (p = 0.085) and HER2+ (p = 0.094), however not reaching statistical significance. High p38 was related to increased relapses within the first years but the difference vanished with longer follow-up time; RFS (p = 0.486) and BCCS (p = 0.589). JNK remained as an independent marker of RFS in a Cox proportional multivariate analysis (HR = 2.19; 95% CI = 1.14-11.41; p = 0.029), together with nodal status (HR = 3.6; 95% CI = 1.44-8.81; p = 0.006) and histological grade (HR = 3.1; 95% CI = 1.29-7.56; p = 0.011). Discussion: Our data on ERK and p38 is concordant with results from previous published studies. Two studies have shown ERK expression, determined by IHC to be correlated with “good prognosis features” as smaller tumour size, absence of nodal metastasis, lower proliferation and a favourable outcome after adjuvant tamoxifen (Svensson et al. 2005, Bergqvist et al. 2006). Higher p38 determined by use of an ELISA has been suggested as a marker of early relapses i.e. intrinsic resistance, although methodological problems must be taken into account (Linderholm et al. 2011). By use of IHC we could confirm that patients with higher expression of p38 actually had significantly more relapses within early follow-up. JNK was the MAPK with the most pronounced effect on survival. Absent or low expression was significantly correlated with an impaired survival in both uni- and multivariate analyses, a finding to our very best knowledge not described before. Low JNK was actually found more frequently in patients with a HER2 positive or Luminal B like BC (ER+, high proliferation rate or HER2+). Conclusions: Our results suggest that MAPK’s can be evaluated by IHC on TMA’s. Absence/low JNK was significantly correlated with an impaired survival and a Luminal B like subtype. Confirming studies in large preferable randomised patient populations are warranted.

P1-06-20
Response to Neo-Adjuvant Chemotherapy and Outcomes for I-SPY 1 Patients Stratified by the 70-Gene Prognosis Signature (MammaPrint) and Molecular Subtyping (BluePrint).
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Background
Classification of breast cancers into molecular subtypes may be important for the proper selection of therapy for patients as tumors with seemingly similar biology can have strikingly different clinical outcomes. We analyzed response to neo-adjuvant chemotherapy as a function of molecular subtypes and show patient survival from the multi-center neo adjuvant trial, I-SPY 1 (CALGB 150007/150012; ACRIN 6657). Previous analyses had shown that breast cancer subtypes as identified by immunohistochemistry or molecular analyses, have distinct clinical outcome (Esserman, ASCO 2009). Here, we analyze how the 70-gene signature (MammaPrint) together with an 80-gene molecular subtyping profile (BluePrint=Basal-type, Luminal-type, ERBB2-type; de Snoo, ASCO, 2010 and manuscript under review) can sub-classify patients.

Materials
This study was carried out on genomics data of core needle biopsies from 149 out of 221 patients enrolled in the I-SPY 1 trial, a multi-center trial designed to identify predictive markers of pathological complete response (pCR) and disease-free survival (DFS) in women with locally advanced breast cancer (3 cm or greater). All women received neoadjuvant Doxorubicin & Cytoxan followed by paclitaxel chemotherapy. The 70-gene and the subtyping signatures, Basal, Luminal and ERBB2-type were determined on 44K Agilent arrays available through the I-SPY 1 data portal (Esserman, ASCO, 2009 and manuscript under review).

Results
The 70-gene signature classified 9% of patients (13/149) as Low Risk, of whom one patient was ERBB2-type, and the other 12 were Luminal-type. None of these patients experienced a pCR. The remaining 136 were classified as 70-gene High Risk (91%), of whom 47% were classified as Luminal-type with a pCR rate of 13%, 41% were Basal-type with a pCR rate of 34%, and 12% were ERBB2-type with a pCR rate of 56%.

Patients with BluePrint Basal-type tumors had a 5-year DFS of 61%; ERBB2-type had a 5-year DFS of 78%; 70-gene High Risk/Luminal-type had a 5-year DFS of 73% and 70-gene Low Risk/Luminal-type showed 5-year DFS of 100%.

The molecular subtype classification shows significant association to clinically assessed receptor status. However, clinically assessed HER2+ patients were distributed across all molecular subtypes, where ER+HER+ are predominantly classified as Luminal-type.

Conclusion
This study was performed with the I-SPY 1 dataset, which provides a platform to compare, contrast & combine marker signatures to tailor therapy. We show how combining BluePrint, with MammaPrint risk-classification can detect specific groups of patients who are at high risk of recurrence and who would have a higher likelihood to benefit from chemotherapy. Furthermore, MammaPrint Low Risk patients do not benefit from neo-adjuvant therapy, though have excellent survival rates, suggesting that the Low Risk Luminal-type patients could be managed conservatively.

P1-06-21
Relationship between Body Mass Index and Preoperative Treatment Response to Aromatase Inhibitor Exemestane in Postmenopausal Patients with Primary Breast Cancer.
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Background:
Some studies have shown that high body mass index (BMI) is associated with inferior outcome after adjuvant therapy with non-stereoidal aromatase inhibitor, anastrozole in breast cancer patients. We aimed to investigate predictive effect of BMI on clinical response to neoadjuvant therapy with steroidal aromatase inhibitor, exemestane in postmenopausal patients with primary breast cancer.

Patients and methods:
The study group consisted of 109 patients from the JFMC 34-0601 neoadjuvant endocrine therapy trial in which postmenopausal patients...
with estrogen receptor (ER)-positive primary breast cancer were administered exemestane (25 mg/day) for 24 weeks before surgery. Patients were categorized into three groups according to BMI: low (BMI < 22 kg/m²), intermediate (22 ≤ BMI < 25 kg/m²) and high (BMI ≥ 25 kg/m²). Statistical analyses were performed to explore the predictive effect of BMI on clinical response using a multivariable logistic regression model.

Results:
Higher BMI correlated with positive progesterone receptor status (p < 0.01) and low Ki-67 index (p = 0.03). Objective response rates (ORR) were 21.7% in low BMI, 56.0% in intermediate BMI and 60.6% in high BMI, respectively (p = 0.01). In a multivariate analysis, only low BMI was an independent negative predictor of clinical response.

Conclusion:
Low BMI was associated with a decreased ORR to neoadjuvant endocrine therapy with exemestane. Our results may suggest that the predictive effect of BMI varies according to the type of aromatase inhibitor and objective outcome.

P1-06-22
Identification of Biomarkers in Breast Cancer for Prediction of Response to PARP Inhibitor Olaparib.

Background: Poly (ADP-ribose) polymerase (PARP) is an enzyme involved in DNA repair. PARP inhibitors operate on the principle of synthetic lethality in conjunction with DNA damaging agents, and are likely to be useful for treatment of BRCA-mutated and triple negative breast cancers exhibiting ‘BRCA-ness’ or other signs of DNA repair deficiency. Multiple PARP inhibitors have been developed, such as Olaparib (AstraZeneca), BSI-201 (Sanofi-Aventis) and ABT-888 (Abbott Laboratories). Though some clinical trials have shown drugs in this class to be promising, not all results have been positive. As PARP inhibitors differ in mechanism of action, dosing interval and toxicities, trial results seem to depend on the specific combination of PARP inhibitor and patient population. To understand why some studies succeeded and others failed and to guide new clinical trials in patient selection, there is an urgent need for biomarker identification, both for PARP inhibitors in general and for the specific idiosyncratic mechanisms of each drug.

Material and Methods: Thirty-three in vitro breast cancer cell lines were administered the PARP inhibitor Olaparib, with sensitivity to the compound summarized as the dose necessary to kill 50% of each culture. mRNA expression (Affymetrix U133A, Exon 1.0ST array) and transcriptome sequence (Illumina GAII) were available for 22/33 cell lines, among which 9 were sensitive and 13 resistant. To obtain robust predictive markers that are minimally dependent on the specific PARP inhibitor and expression platform, a bottom-up approach was opted for, restricted to genes in the major DNA repair pathways. Logistic regression with forward selection was used to determine the most important markers, further reduced based on consistency across platforms. The weighted voting algorithm was used to build the final predictor. Eight U133A data sets with number of tumor samples varying from 61 to 289 were subsequently used to verify prevalence and to identify the subpopulations that are likely to respond according to the predictor. To verify cross-platform generalizability, the signature was additionally tested in 430 TCGA samples with custom Agilent 244K gene expression.

Results: For the development of a genomic signature that might work for multiple PARP inhibitors and expression platforms, prior knowledge of DNA repair pathways was incorporated and stringent criteria for marker inclusion were applied using three different platforms. Eight genes fulfilled the criteria, of which 5 were resistance markers and 3 sensitivity markers. When testing the 8-gene signature in eight U133A data sets, 40-48% of patients were predicted to be responsive to Olaparib. In addition, well-known markers (ER, PR, ERBB2, KRT5/17) were examined in patients expressing the 8-gene sensitivity signature. A higher percentage of these patients were ERBB2-negative and did overexpress KRT5/17, indicative for the basal subtype. Prevalence and relationship with these markers were confirmed in 430 samples on a distinct platform (Agilent).

Conclusion: Cell line exposure to Olaparib has yielded an 8-gene predictor of sensitivity. This signature was observed in a substantial fraction of primary breast tumors predicted to benefit from Olaparib.

P1-06-23
Sprecher E, Lezon-Geyda K, Sarkar S, Bossuyt V, Narayaan M, Kumar A, Krop I, Winer E, Tuck D, Kleinstein S, Harris L, Yale University, New Haven, CT; Yale University; Beckman Coulter; Stony Brook University Hospital, Stony Brook, NY; Dana-Farber Cancer Institute, Boston, MA

Introduction: Trastuzumab is a targeted therapy against the HER2 cell surface receptor and has greatly improved prognosis for HER2+ breast cancer patients. Despite decades of research, the mechanism of action of T remains unclear and mechanisms of resistance have not been adequately defined. We sought to determine if models of response and resistance in vitro could identify biomarkers in vivo in HER2+ early breast cancer.

Methods: BT474 and UACC812 cell lines were treated with 10ug/ml T for 0 and 24 hours. Fresh tumor core biopsies were taken at a 2 week timepoint after a single dose of T (8mg/m2) from 80 HER2-overexpressing, early breast cancer patients enrolled on a clinical trial of T>T+C. Nucleic acids were extracted using Qiagen AllPrep and were analyzed with Illumina HT12v3 Beadchip arrays. All arrays were processed at the Yale Center for Genome Analysis (West Haven, CT). Clinical response at surgery was defined as pathologic complete response (pCR), objective response (CR+PR=OR) and non-response (SD+PD=NOR) by RECIST criteria. Gene expression was analyzed in Bioconductor using LIMMA analysis. Pathway analysis was performed using the DAVID bioinformatics resource.

Results: We identified gene expression signatures of T response and resistance in HER2+ breast cancer cell lines across 24-hour exposure to trastuzumab. 180-genes changed significantly in a T-sensitive HER2+ cell line (BT474) and 58 genes changed significantly across treatment in a T-resistant HER2+ cell line (UACC812). We applied these signatures to gene expression profiles from early stage HER2+ breast tumors treated with a single dose of T. The BT474 T-responsive signature was enriched among the changes in expression across treatment for responsive patients. The expression change for the BT474 signature genes was also able to partially cluster responsive and resistant breast tumors by outcome. A subset of the UACC812 T-resistance signature was also enriched in the differential between responsive and resistant tumors prior to T treatment.
Pathway analysis based on the direction of change of genes in pCR and sensitive (BT474) cell lines found gland development/differentiation (enrichment score=4.5), DNA synthesis (ES=4.0), chaperone (ES=2.8) and transcriptional machinery (ES=2.2) to be coordinately downregulated in both sensitive cell lines and tumors. This suggests that the downregulation of differentiation pathways seen in our ‘pCR signature’ (Harris et al: AACR 2011) is not an epiphenomenon of cell loss. There were no significant pathways upregulated in both sensitive tumors and cell lines, however discordant genes were enriched in chromatin regulation pathways (ES=4.1). Of note, our previous findings of amplicon gene downregulation in pCR tumors, yet upregulation in cell lines points to a novel mechanism of chromatin modulation heretofor undiscovered in response to T.

Conclusions: These results demonstrate the value of iterative study of in vitro and in vivo response mechanisms in HER2 cell lines and tumors, and the importance of brief exposure studies in understanding the mechanism of response to T, and other targeted therapies.

**P1-06-24**

**Nuclear Localization of Stat5a Predicts Response to Antiestrogen Therapy and Prognosis of Clinical Breast Cancer Outcome.**

Peck AR, Witkiewicz AK, Liu C, Klimowicz AC, Stringer GA, Pequignot E, Freydin B, Yang N, Tran TH, Rosenberg AL, Hooke JA, Kovatch AJ, Shriver CD, Rimm DL, Magliocco AM, Hyslop T, Rau H. Thomas Jefferson University, Philadelphia, PA; Tom Baker Cancer Center, Calgary, AB, Canada; Walter Reed Army Medical Center, Washington, DC; MDR Global Systems, LLC, Windber, PA; Yale University School of Medicine, New Haven, CT

Nuclear-localized and tyrosine-phosphorylated Stat5 has been reported as a favorable prognostic marker and predictor of response to antiestrogen therapy in breast cancer. Phospho-Stat5 antibodies do not distinguish between phosphorylated Stat5a and the closely related Stat5b, but Stat5a is considered more critical for normal mammary development than Stat5b. The purpose of this study was to determine whether levels of nuclear-localized Stat5a protein (Nuc-Stat5a) were prognostic of clinical outcome or predictive of antiestrogen response. Stat5a was detected by traditional diaminobenzidine-chromogen immunohistochemistry (IHC) and pathologist scoring or by quantitative immunofluorescence in five archival cohorts of breast cancer. Levels of nuclear-localized Stat5a (Nuc-Stat5a) were evaluated by pathologist scoring of whole tissue sections detected by IHC or automated quantitative analysis (AQUA) of immunofluorescently-labeled tissue microarrays. Levels of Nuc-Stat5a were reduced in invasive breast cancer tissues and lymph node metastases compared to normal tissue and ductal carcinoma in situ when quantified by AQUA (Material I; n=180). Tissues from patients not treated with adjuvant therapy or treated with antiestrogen monotherapy were analyzed according to Nuc-Stat5a status for breast cancer-specific survival (CSS) and time to recurrence (TTR) using univariate and multivariate statistical models, adjusting for clinical features including tumor grade, size, lymph node and ER, PR and Her2 status. In two prognostic cohorts of node-negative breast cancer patients, low expression of Nuc-Stat5a detected by standard IHC (Material II; n=223) or quantitative analysis (Material III; n=198), was prognostic of poor breast cancer outcome as measured by univariate and multivariate CSS (Material II/III) and TTR (Material II). CSS and TTR analysis of two independent materials of tumors from patients treated with antiestrogen monotherapy and analyzed by standard IHC (Material IV; n=73) or quantitative immunofluorescence (Material V; n=97) indicated that patients whose tumors expressed low levels of Nuc-Stat5a were at a greater than 4-fold risk of antiestrogen therapy failure when adjusted for hormone receptor status and clinical features (multivariate CSS: Material IV HR=4.3 (1.2,15.6), p=0.03; Material V HR=5.0 (1.87,13.06), p=0.001). In conclusion, loss of Nuc-Stat5a is a promising independent marker of poor breast cancer prognosis in node-negative, non-adjuvant treated breast cancer patients. Additionally, Nuc-Stat5a may be a useful clinical tool to predict tumor response to antiestrogen therapy.

**P1-06-25**

**Changes in FDG PET SUV Correlates with Ki-67 Following 2 Weeks of Aromatase Inhibitor Therapy in ER+ Early Stage Breast Cancer, a Pilot Imaging Study.**

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**Background:** In estrogen receptor positive (ER+) tumors, a low proliferative index (Ki-67) two weeks into endocrine therapy predicts response. FDG PET non-invasively measures tumor sites in vivo. The pre-operative window is an opportunity to assess impact of systemic therapies. We tested associations between FDG PET standardized uptake value (SUV) and Ki-67 after two weeks of aromatase inhibitor (AI) therapy in newly diagnosed, postmenopausal women.

**Methods:** Postmenopausal patients with clinical stage I-II ER+ HER2 primary tumors underwent a 9-35 day “run-in” of AI monotherapy prior to definitive surgery. FDG PET was performed before AI therapy, and 1-5 days before surgery. Ki-67 was measured in baseline core biopsy and surgical specimens.

**Results:** To date, 18 patients (median age 59) have been enrolled of whom 14 patients have undergone serial FDG PET imaging with 12 completed assessment of Ki-67 in surgical samples including one who had no residual invasive carcinoma. The majority harbored ductal carcinomas (n=16) with 10/18 having histologic grade ≥ 2. The median number of days exposed to AI was 18 (range, 9-35). Baseline SUV ranged from 1.8 to 10.9 (median 2.5), and post run-in SUV (7-34 days later) ranged from 1.0 to 10.7 (median 2.5). A median 14% decrease in SUV was observed between paired FDG PET studies (range, 44% decline to 13% increase). Five of 12 patients’ index lesion FDG SUV declined by 20% or more; all also had Ki-67 ≤5% at surgery. An additional 5 patients with Ki-67 ≤5% at surgery had percentage change in FDG PET SUV of 0% to 17% decline. Results will be updated as accrual is ongoing.

**Conclusions:** Substantial changes in FDG PET SUV in the breast tumor were appreciated early in AI therapy. SUV declined or was stable in all but one patient of 14, and all patients with ≤20% decrease in SUV had a low ≤5% Ki-67 at surgery. Serial PET is a promising measure of early response to therapy.
P1-06-26
The EndoPredict Score Is a Response Predictor for Neoadjuvant Chemotherapy in ER-Positive, HER2-Negative Breast Cancer.
Brase JC, Gehrmann MC, Petry C, Weber KE, Schmidt M, Köhl H, Brauch H, Schwab M, Müller V, Jänicke F, Rody A, Kaufmann M, Filipits M, Gnant M, Denkert C, Loibl S, von Minckwitz G, Kronenwett R. Sividon Diagnostics, Cologne, Germany; Bayer Technology Services GmbH, Leverkusen, Germany; University of Mainz, Germany; Dr. Margaretre Fischer-Bosch Institute of Clinical Pharmacology Stuttgart and University Tübingen, Germany; University Medical Center Hamburg-Eppendorf, Germany; J.W. Goethe University, Frankfurt, Germany; Medical University of Vienna, Austria; Charité - University of Berlin, Germany; German Breast Group, Neu-Isernburg, Germany

Background:
The EndoPredict (EP) score is a multigene classifier to predict the likelihood of distant recurrence in ER-positive, HER2-negative breast cancer patients treated with adjuvant endocrine therapy. Two large randomized phase III trials involving endocrine therapy only (n > 1700) demonstrated additional prognostic information of the EP score independent from clinicopathological parameters by classifying 49% as low risk. However, the predictive role of the EP is not clear. Therefore, we examined whether the EP Score also predicts sensitivity towards neoadjuvant chemotherapy in ER-positive, HER2-negative breast cancer patients.

Methods:
Four publicly available gene expression data sets (Affymetrix HG-U133A) were retrieved from the gene expression omnibus (GEO) data repository. All analyzed breast cancer patients were treated with anthracycline or taxane/anthracycline-based neoadjuvant chemotherapy. Microarray cel files were MAS5 normalized with a global scaling procedure and a target intensity of 500. The analysis was restricted to ER-positive, HER2-negative breast cancer patients according to pre-specified cut-off levels for the respective ESR1/ERBB2 Affymetrix probesets. The EP score was calculated and patients were classified as having low or high risk according to the pre-specified validated cut-off value. Pathological complete response (pCR) - defined as no residual invasive cancer in the breast or lymph nodes - was used as the primary endpoint for the assessment of treatment response.

Results:
The EP Score was examined in 221 ER-positive, HER2-negative breast cancer patients treated with neoadjuvant therapy. Among the 221 patients, 61 tumors (27.6%) were classified as EP-low-risk, whereas 160 tumors (72.4%) were EP-high-risk. Only one of the EP-low-risk tumors achieved a pCR after neoadjuvant therapy, whereas 24 of the 25 pCR events were classified as EP high risk. The sensitivity of the EP score was 96% and the negative predictive value 98% with an area under the receiver operating characteristic curve of 0.73.

Conclusions:
The EP Score is a predictor of chemosensitivity in the neoadjuvant setting. The test correctly identified all but one of the patients achieving a pCR suggesting that the benefit of cytotoxic chemotherapy is limited to the EP high risk group.

P1-07-01
Comparison of Four HER2 Testing Methods in the Detection of HER2-Positive Breast Cancer: Results from the FinHer Study Cohort.
Huang W, Wirtz R, Weidler J, Lie Y, Sherwood T, Leinonen M, Bono P, Isola J, Kellokumpu-Lehtinen P-L, Joensuu H. Monogram Biosciences Inc., So. San Francisco, CA; STRATIFYER Molecular Pathology GmbH, Cologne, Germany; Pharma, Turku, Finland; Helsinki University Central Hospital, Helsinki, Finland; Institute of Medical Technology, University of Tampere and Tampere University Hospital, Tampere, Finland; Tampere University Hospital, Tampere, Finland

Background: Accurate assessment of the HER2 status is essential for identifying patients who may benefit from HER2 targeted therapy. The current methods, immunohistochemistry (IHC) and in situ hybridization (ISH), determine HER2 status semi-quantitatively as positive (+), equivocal (+/-) and negative (-) with predefined cutoff values. Recent studies have suggested that current HER2 cutoffs may not be optimal for all clinical settings of HER2 targeted therapy. In a small subset of adjuvant NCCTG N9831 patients confirmed as HER2-normal by round-robins of HER2 testing, trastuzumab benefit was observed (Perez et al, SABCS 2010). Quantification of HER2 as continuous variable may enable a more accurate optimization of HER2 cutoffs for various HER2 targeted therapies. In this study, we measured continuous HER2 protein expression by the HERmark™ assay and continuous mRNA expression by quantitative real time polymerase chain reaction (qPCR), and compared these results with central IHC and central chromogenic in situ hybridization (CISH) results of FinHer.

Methods: Total HER2 protein expression (H2T) was quantified using the HERmark assay as previously described (Huang et al. Am J Clin Pathol 2010;134:303). HER2 mRNA expression (H2N) was measured by qPCR as previously published (Noske et al. Br Cancer Res Treat 2011;126:109). The results of H2T and H2N as continuous variables and as predefined categories were compared with central CISH results from FinHer (Joensuu et al, N Engl J Med 2006;354), and central IHC retesting.

Results: H2T in 899 evaluable samples described a continuum of 0.4 to 721.2 (relative HERmark unit); while H2N in 915 evaluable samples showed a continuum of 31.4 to 42.8 (delta-Ct). Significant correlation between H2T and H2N as continuous variable was found (R² = 0.56, Pc < .0001). Paired method comparison was performed for samples with valid results in any two of the four testing methods. Overall concordance of H2T and H2N with predefined categories (+, +/-, -) was 81%, and concordance of (+) and (-) subsets was 95% when (+/-) cases (H2T 11%; H2N 6%) were excluded. Overall concordance of central IHC and H2T categories (+, +/-, -) was 75%, and concordance of (+) and (-) subsets was 96% when (+/-) cases (IHC 16%; H2T 11%) were excluded. Overall concordance of IHC and H2N categories (+, +/-, -) was 84%, and concordance of (+) and (-) subsets was 99% when (+/-) cases (IHC 16%; H2N 6%) were excluded. Concordance of central CISH (+, -) with H2T and H2N categories (+, -) was 89% and 91%, respectively, when (+/-) cases were excluded from H2T (13%) and H2N (8%), respectively.

Conclusions: All four methods identified HER2-positive breast cancers. The discordance rate between the methods tested was approximately 10 to 20% despite careful delineation of cancerous tissue in the sample and analysis of adjacent tumor sections. No combination of assays could be identified with concordance rate >95% when the equivocal subsets were included in comparisons. Exclusion of the equivocal subsets (about 10% of samples) yielded
Discordance between Central and Local Laboratory HER2 Testing from a Large HER2-Negative Population in VIRGO, a Metastatic Breast Cancer Registry.

Vogel CL, Bloom K, Burris H, Gralow JR, Mayer M, Pegram M, Rugo HS, Swain SM, Yardley DA, Chau M, Lalla D, Brummer MG, Kaufman PA. Sylvester Comprehensive Center at Deersfield, Miller School of Medicine, University of Miami, Miami, FL; Clariant, Inc., Aliso Viejo, CA; Sarah Cannon Research Institute, Nashville, TN; Tennessee Oncology, PLLC, Nashville, TN; University of Washington Medical Oncology, Seattle Cancer Care Alliance, Seattle, WA; Patient Advocate, New York, NY; University of California San Francisco Comprehensive Cancer Center, San Francisco, CA; Washington Cancer Institute, Washington Hospital Center, Washington, DC; Genentech, Inc., South San Francisco, CA; Norris Cotton Cancer Center; Dartmouth-Hitchcock Medical Center, Lebanon, NH

Background: HER2 overexpression is associated with unfavorable prognosis and is reported in 18–25% of breast cancers (BC). HER2 testing is often performed using immunohistochemistry (IHC) or fluorescence in situ hybridization (FISH). Because of the significant benefit of HER2-directed therapies, it is critical to accurately identify women whose tumors are HER2+. Reports have noted discordance between HER2+ test results from local vs. reference labs in patients with HER2+ BC evaluated for trastuzumab-based clinical trials. There are little published data on central testing of BC found to be negative locally.

Patients and Methods: VIRGO is an observational cohort of N=1,287 women with primarily HER2-negative metastatic BC. An optional tissue collection substudy was conducted, and 776 patients were included in the primary analysis. Central testing was performed at 2 reference labs and tumors were deemed HER2+ if IHC 3+ and/or FISH positive (HER2:CEP17 ratio ≥2.0). Tumors with unknown/missing local HER2 status (n=68) were excluded from primary analyses. Number of patients potentially affected based on BC incidences from the American Cancer Society (ACS) 2011 estimates and the World Health Organization (WHO) 2008 report were calculated. Testing on the remaining HER2-negative cohort is in process.

Results: Central retesting has been performed on tumor samples from n=373 patients to date. HER2-negative locally evaluable tumors (n=301), n=4 HER2-negative locally with no evaluable tumor, and HER2 unknown (n=68). A total of 301 unique patient samples were included in the primary analysis. Of these, 15 (4.98% [95% CI (2.7%, 7.9%)]) were found to be HER2+ by central testing (Table). Based on sensitivity analyses assuming all 68 tumors with unknown HER2 status to be negative locally, 4.07% (15/369) would be centrally HER2+. Of the 15 HER2+ tumors, 4 tumors tested positive centrally by both IHC and FISH; 6 IHC positive/FISH negative; and 5 FISH positive/IHC negative. 14/15 tumors were tested centrally by only one testing methodology, and 11/15 were determined to be HER2+ centrally based on the testing methodology not performed locally. Investigators for all 15 patients have been notified of central HER2 testing results.

Conclusion: Based on ACS estimates of 232,620 new cases of invasive BC diagnosed in the US in 2011 (assuming 80% testing HER2-negative); a discordance rate of 4.5% equates to 7,444–9,305 patients’ tumors diagnosed as HER2+ by central testing. Based on WHO global BC incidence estimates, 44,274–55,342 patients could be impacted worldwide as reported in this study. Inaccurate HER2 testing has significant clinical impact, both in denying appropriate treatment or leading to inappropriate use of HER2-targeted therapies. This study suggests testing by both IHC and FISH may be of benefit to accurately identify HER2 status, consistent with the Herceptin® USPI.
moderate effects that appear less critical to measurement accuracy than the issue of tumor heterogeneity. We identified 4 proteins which show a significant change with increasing time to formalin fixation and should allow construction of a TQI for assessment of pre-analytic antigenic degradation.

P1-07-04
Gene Expression Module Biomarkers To Stratify Multiple Clinical and Therapeutic Endpoints for Universal Breast Cancer Companion Diagnostic.

Gene expression patterns are increasingly capable of stratifying patients based on prognosis and response to therapy. Given the limited availability of sample tissue, however, it is not feasible to run many tests, suggesting the need for a universal companion diagnostic assay that is informative with respect to multiple clinical and therapeutic endpoints. Key challenges are identification of appropriate gene expression biomarkers, translation of biomarkers to clinical assays, and development of reliable gene expression profiling of formalin-fixed clinical specimens. Here, we describe a meta-analysis approach that identifies novel biomarker modules that results in multiple clinical and therapeutic read-outs.

A co-expression meta-analysis of 5,339 breast tumors from 56 microarray datasets identified highly co-expressed sets of genes (modules) across multiple datasets. These module based biomarkers were tested for their ability to associate with prognostic and predictive targets in published datasets. In addition, each module was reduced from 10 – 1,000 genes to the top performing 2-3 genes based on the degree of co-expression across the meta-analysis and validation by quantitative PCR in an independent panel of FFPE tumor samples. This study demonstrates that a single 96 gene qPCR test utilizing multiple module biomarkers is not only capable of stratifying patients by standard histopathological parameters (ER, PR and Her2), but also stratifies by other diverse elements of the disease (cell lineage, dysregulated core biological functions, factors of cell growth, underlying genomic aberrations and the tumor microenvironment). Taken together, these biological variables represent the major biological diversity present within the breast cancer population. A series of retrospective analyses demonstrated that different single module and combinations of modules were capable of predicting a variety of clinical endpoints, including 5-year survival, neoadjuvant chemotherapy response in ER-patients and targeted therapy response in model systems. The molecular heterogeneity of breast cancer can be summarized by discrete gene expression modules that individually represent distinct biological pathways, and that collectively can be represented by as few as 96 genes. These breast cancer modules, together with outlier genes, allow for summarization of the entire transcriptional program and provide a universal assay with broad application to companion diagnostics development.

P1-07-05
HER2 Status Resolution in FISH and IHC “Double Equivocal” Breast Carcinomas by Quantitative Real-Time PCR.
Portier BP, Wang Z, Downs-Kelly E, Budd GT, Lanigan C, Tubbs RR. Cleveland Clinic, Cleveland, OH; Taussig Cancer Center; Cleveland Clinic, Cleveland, OH

Background:
Clinical testing for HER2 amplification/over-expression is performed by immunohistochemistry (IHC) and/or fluorescence in situ hybridization (FISH) as outlined by the ASCO/CAP guidelines. Although these guidelines standardize testing and reporting, in a subset of patients, HER2 is equivocal by both IHC and FISH (“Double Equivocal”). These double equivocal patients represent a clinically problematic sub-group that currently lack standardized management guidelines. In this study, we utilize Quantitative Real-Time PCR (Q-RT-PCR) to resolve HER2 status in invasive breast cancers that could not be resolved via IHC and FISH testing.

Material and Methods:
FISH for HER2 was performed on 2259 invasive breast carcinomas from 1/2008 to 12/2010. In accordance with ASCO/CAP, all equivocal HER2 FISH cases were reflex tested by IHC. In double equivocal cases, RNA extraction was performed following macro-dissection using High Pure RNA Paraffin Kit (Roche Applied Biosciences, Indianapolis, IN). Q-RT-PCR was carried out using TaqMan® RNA-to-CT™ 1-Step Kit with primers and probes for HER2, B2M, and GAPDH (Applied Biosystems, Foster City, CA). Q-RT-PCR results were expressed as the relative quantification of HER2 vs. two control genes, all normalized against calibrator RNA from the MCF7 cell line. Cut off for Q-RT-PCR HER2 overexpression was set using ROC curve analysis (MedCalc, Belgium).

Results:
In our cohort of 2259 patients, 124 (5.5%) had an equivocal HER2 result by primary FISH testing. Reflex HER2 testing by IHC was unable to resolve the HER2 status in 35 (1.5%) patients. Detection of HER2 overexpression by Q-RT-PCR was validated using 50 FISH confirmed amplified and 50 non-amplified cases. Q-RT-PCR performed on these 2 control populations generated two non-overlapping populations and ROC curve analysis using a cut off value of 7.0 showed 100% sensitivity and specificity in detection of HER2 overexpression. Application of Q-RT-PCR in the double equivocal sub-group resulted in resolution of HER2 status in all cases, 8 HER2 positive (test value ranging from 7.12 – 15.37) and 14 HER2 negative (test value ranging from 1.05 – 6.92).

Conclusion:
Application of Q-RT-PCR for HER2 represents a viable approach to resolve HER2 status in cases that fail classification by both FISH and IHC. Q-RT-PCR combines the precision and high sensitivity of real-time PCR with the morphological specificity of histological evaluation and ultimately allows definitive HER2 classification at the time of initial diagnosis. This knowledge of HER2 status at the time of diagnosis allows for comprehensive neoadjuvant treatment although, additional studies correlating response to anti-HER2 therapy and HER2 status by Q-RT-PCR are warranted.
PI-07-06

High Concordance of Protein (by IHC), Gene (by FISH; HER-2 Only) and Microarray Readout (by TargetPrint) of ER/PR/HER2: Results from the MINDACT Trial.

Viale G, Bogaerts J, van 't Veer L, Rutgers E, Piccart M, de Snoo F, Engelen K, Russo L, Dell’Orto P, Glas A, Cardoso F. On Behalf of the TRANSBIG Consortium & the MINDACT Investigators. European Institute of Oncology, Milan, Italy; European Organisation of Research and Treatment of Cancer; Brussels, Belgium; Netherlands Cancer Institute, Amsterdam, Netherlands; Institute Jules Bordet, Brussels, Belgium; AgendiaNV, Amsterdam, Netherlands; Champalimaud Cancer Center, Lisboa, Portugal

Background

Previously, the micro-array readout of ER, PR and HER2 by TargetPrint was shown to be strongly correlated with high quality immunohistochemistry (IHC)/FISH assessment, especially for ER and HER2. Concordance rates were 93% (k=0.79) for ER; 83% (k=0.65) for PR and 96% for HER2 (k=0.88) in 636 patients (Roepman et al., Clin Cancer Res, 2009). This study analysis was undertaken to further determine the correlation of microarray readout with IHC/FISH assessment both locally and centrally determined in the first 800 pts enrolled in the MINDACT trial. This work is essential to determine the quality of biological data in the two risk assessment methods used in MINDACT based upon which adjuvant chemotherapy decision is made, in order to exclude bias.

Methods

ER/PR/HER2 IHC assessment was performed on the first 800 primary breast cancers (BC) of pts enrolled in the MINDACT study. The assessment was performed locally at each center (n=800) and by central review at the laboratory of the European Institute of Oncology (n=626). A tumor was classified positive for ER and PR when 1% of tumor cells showed positive staining. HER2 IHC status was scored as 0, 1+, 2+ or 3+. A score of 3+ was considered positive. In 2+ cases FISH was performed to assess final HER2 status. Gene expression data for ER, PR and HER2 were obtained by TargetPrint stratified based upon which adjuvant chemotherapy decision is made, in order to exclude bias.

Results

Comparison of local assessment (IHC & FISH for HER2) with central review indicated highly similar results for receptor readout with a concordance of 98% (k=0.90) for ER; and 96% for HER2 (k=0.80) and slightly lower for PR (90% (k=0.72)). Comparison of central assessment (IHC & FISH for HER2) with micro array readout by TargetPrint indicated highly similar results for receptor readout with a concordance of 97% (k=0.88) for ER and 95% for HER2 (k=0.76). For PR the concordance was lower but still quite acceptable (85% (k=0.62)).

Conclusion

Local and centrally assessed ER, PR and HER2 status in the first 800 MINDACT patient samples indicate a high level of quality for pathology in the local participating hospitals. These results exclude any bias induced by a lower quality of “traditional” pathology results as compared to the centrally assessed MammaPrint, both used for risk assessment and adjuvant chemotherapy decision in the MINDACT trial. The microarray-based assessment of ER, PR and HER2 gives results comparable to IHC & FISH and provides an objective and quantitative assessment of tumor receptor status. These results indicate that TargetPrint can serve as a second pathology assessment for locally assessed parameters, especially since TargetPrint is part of a multi-profile platform for breast cancer treatment management. This work was funded by the Breast Cancer Research Foundation and the EU Framework Program VI.

PI-07-07

Assessing Two Methods of Meta-Analysis in Studies of Patients with Breast Cancer: Individual Patient Data-Based (IPD) Versus Literature Based Abstracted Data (AD) in 5 Meta-Analyses Including over 28,000 Patients. Are There Results Differences of Concern?

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Background: Meta-analyses are widely used to provide high level evidence to resolve controversies. Differences of opinion continue concerning the two most frequently used methods (IPD and AD) and their roles in providing reliable recommendations for Evidence-Based Medicine. Some regard the IPD method as being more accurate, yet it is time-consuming, expensive, and can be troubled by getting data from all investigators. We tested whether overall conclusions would differ between the two methods when using the same studies for comparison. To overcome limitations related to simple correlation analysis, and: 1) to weigh the agreement between these 2 approaches (Bland-Altman [BA], Lancet 1986), and 2) to evaluate the eventual predictive role of the data coming from AD over IPD, an analysis with multiple methods was performed.

Methods: 5 meta-analyses in breast cancer examining 9 outcomes which had been subjected to IPD meta-analysis were selected. Literature-based AD meta-analyses were then conducted. Hazard Ratios (HR) and Risk Ratios (RRs) with 95% confidence intervals (CI) were extracted or derived from original meta-analyses. At least 90% of patients’ reports were required for the IPD analysis. Methods agreement was analyzed according to the BA method, as differences or ratios, and a comparison with the Variance-Ratio test (F-test), and the Concordance Correlation Coefficient (CCC) was done. Correlations between IPD/AD-ratios were estimated using a linear regression model with Pearson (r) and R² coefficients (parametric) and Spearman’s Rho/Kendall’s Tau coefficient (non-parametric), to derive a predictive estimation on IPD when adopting AD. MedCalc® Software was used.

Results: 9 outcomes in 5 meta-analyses (28,358 patients) were analyzed, as seen in the table.

Graphical comparison between the deviation from the ‘line of identity’ identified with the B-A method did not show meaningful differences between AD and IPD (mean difference -0.01535, 95% CI -0.0315, 0.0008; mean ratio 0.9816, 95% CI 0.9625, 1.0008). No significant difference in the Variance (F-test 1.01, p=0.98), and a high concordance (CCC 0.9775, 95% CI 0.91, 0.99) were found. A highly significant correlation by both parametric and non-parametric tests allowed derivation of a linear regression equation able to predict estimation IPD results from AD data; R² = 0.97, p<0.001.

Conclusions: When analyzing the results of studies (AD) or patient results from those same studies (IDP) we found: 1) No significant or
meaningful differences between the 2 meta-analysis methods; and 2) Data from the AD analyses are able to predict those from IPD, without significant risk of overestimation.

**P1-07-08**


Background: Genomic grade (GG) is a 97-gene signature which improves the accuracy and prognostic value of histological grade (HG) in invasive breast cancer (IBC) (Sotiriou JNCI 2006). It is particularly useful in stratifying HG2 tumors into GG-1 (low grade) and GG-3 (high grade) tumors. In order to extend the applicability of GG on routinely-used formalin-fixed-paraffin-embedded (FFPE) samples, the original microarray MapQuant Dx® signature was converted into a RQ-PCR assay (PCR-GG). The study aimed at validating the grading classification performance of the newly developed PCR-GG test.

Methods: Subsets of genes derived from the original GG were selected by testing their grading classification and prognostic performances using different classifiers on 15 independent public microarray data sets. 25 most-performing genes were quantified by RQ-PCR using a TaqMan assay on a training set consisting of 91 early IBC cases graded acc. to the Elston-Ellis recommendations, and classification performance of various combinations assessed.

Analytical performance of the final reduced signature was assessed on 4 samples (5 RNA extractions and 3 RQ-PCR per sample) and used to define a 95% CI around the GG-1/GG-3 cut-off. Cases with a PCR-GG score falling into the 95% CI were considered “Equivocal” (Eq). Concordance with the MapQuant Dx® GG was assessed on 44 paired FFPE/frozen samples of the training set. Grade classification performance of the PCR-GG test was evaluated on an independent validation set of 396 FFPE samples retrieved from the Department of Pathology (2004 to 2010) of Institut Jules Bordet (Brussels, Be). FFPE samples from early invasive, NO, ER+, HER2-, ductal or lobular BC containing ≥30% inv. tumor cells were selected. Classification performance was evaluated on the whole cohort and by histological subtype.

Results: The PCR-GG signature is based on the expression of 6 reporter and 3 reference genes. Most of the genes are overexpressed in grade 3 tumors and those associated with proliferation. The 9-gene PCR-GG showed a high concordance with the original GG microarray test (95%). 388 eligible cases were available for validation. 336 samples contained ≥30% inv. tumor cells and 322 were successfully amplified, corresponding to a 96% technical success rate. The HG1, HG2 and HG3 distribution in the validation cohort were 27%, 55% and 18% resp. PCR-GG reclassified 84% of all tumors (Table 1) with an overall concordance of 90% (92% HG1/GG-1 concordance and 88% HG3/GG-3). 82% of HG2 tumors were reclassified into 74% GG-1 and 26% GG-3. Overall concordance was 91% in the Ductal subset (comprising 84% of all cases) and 89% in the Lobular subset.

Conclusion: The newly developed PCR-GG test composed of 9 genes will open its use in routine clinical practice.

**P1-07-09**

Estrogen Receptor (ER) mRNA and ER-Related Gene Expression in Breast Cancers That Are 1%-10% ER-Positive by Immunohistochemistry.

Iwamoto T, Booser D, Valero V, Murray JL, Koening K, Esteva FJ, Ueno NT, Zhang J, Shi W, Qi Y, Matsuoka J, Hortobagyi GN, Hatzis C, Symmans WF, Paszti E, The University of Texas MD Anderson Cancer Center, TX; Okayama University, Okayama, Japan; Nivera Biosciences Inc, MA

Purpose: Our goal was to examine whether borderline estrogen receptor (ER)-positive cancers, defined as 1-10% positivity by immunohistochemistry (IHC), show the same global gene expression pattern and high ESR1 mRNA expression as ER-positive cancers or are more similar to ER-negative cancers.

Patients and methods: ER status was determined by IHC in 465 primary breast cancers and with Affymetrix U133A gene chip (ESR1 mRNA gene expression: Probe set = 205255_at). We compared expressions of ESR1 mRNA and a 106-probe set ER-associated gene signature score between ER-negative (n=183), 1-9% (n=25), exactly 10% (n=6), and > 10% ER-positive (n=251) cancers. We also assessed the molecular class of the borderline ER-positive cases using the PAM-50 classifier.

Results: Among the 1-9%, 10% and > 10% IHC positive cases, 24%, 67% and 92% were also ER-positive by ESR1 mRNA expression. The average ESR1 expression was significantly higher in the > 10% IHC-positive cohorts compared to the 1-9% or completely negative cases but in these latter two cohorts ER expression was similarly low. The average ER gene signature scores were similar for the ER-negative and 1-9% IHC-positive cases, but significantly lower than in > 10% ER-positive cases. None of the 1-9% ER-positive cases were classified as Luminal A, 2 were Luminal B and 12 were Basal-like. Among the 10% ER-positive cases, 2 were Luminal A and 1 was Luminal B. Conclusion: Overall, 24% of the 1-9% and 67% of the 10% ER-positive cancers show ESR1 mRNA levels and gene signatures that are consistent with ER-positive, potentially endocrine sensitive tumors.

**P1-07-10**

Validation of a Diagnostic Molecular Signature (EHT Dxl4) on Fine-Needle Aspirate Samples from Breast Tumors.

Delaloge S, André F, Fehlbaum P, Sol O, Vielh P, Institut Gustave Roussy, Villejuif, France; Exonhit, Paris, France

Background: A 1228-probeset molecular classifier (EHT Dxl4) able to discriminate benign breast lesions from breast adenocarcinoma with a 96% performance was previously identified in a panel of 71 fine-needle aspirate (FNA) samples of nodular breast lesions collected at Institut Gustave Roussy (IGR). The microarray which enabled the signature identification was based on the genomewide RNA analysis using the ExonHit’s SpliceArray™ technology. The objective of the current study was to validate the performance of the EHT Dxl4 molecular classifier on an independent cohort of FNA samples stored at the Centre de Ressources Biologiques at IGR.

Patients and methods: The samples were collected from women undergoing investigations for suspicious nodular breast lesions classified by ICDR ACR 4 or 5 on mammogram and/or ultrasonography. The morphological cytological analysis of FNA samples classified the
lesions as either benign, malignant or indeterminate. All samples were associated with a definite clinico-pathological diagnosis of the breast lesions. The first part of the study aims at confirming the sensitivity, specificity and overall performance of EHT Dx14 among lesions clearly classified by morphological cytology. The second part aims at establishing its performance among lesions with indeterminate cytological results.

Results: In the first part of the study, the EHT Dx14 transcriptomic signature was applied on a set of 94 FNA consecutive samples categorized as benign (n=47) or adenocarcinoma (n=47) on cytology. The benign/malignant status of the lesions was confirmed by subsequent investigations or follow-up. Results of EHT DX-14 assessment of the samples were respectively 97.9% (95%CI: [88.7%-99.9%]) for sensitivity and 91.5% (95%CI: [79.6%-97.6%]) for specificity. Results on indeterminate FNA samples are being investigated in the second part of the study.

Conclusion: This study did confirm the high performance of EHT Dx14 signature in identifying malignant from benign breast lesions in clinically suspect lesions. EHT Dx14 may provide adequate biological diagnosis in the absence of capability of morphological evaluation.

P1-07-11
Consistency and Control in Clinical Assay Technology over Time: The Oncotype DX Recurrence Score and Assessment of Single Gene Expression Levels.


Background: ASCO® and CAP have recently highlighted the importance of consistency and control in clinical assay technology. To date the Oncotype DX® Recurrence Score® (RS) has been ordered to assist in individualized treatment decision making in over 200,000 estrogen receptor positive, early stage breast cancer patients. The assay quantifies gene expression using RT-PCR from fixed paraffin embedded tissue (FPET) and employs a large number of controls and calibrators to enhance precision and reproducibility. Over 6 years of data on the assay presents an opportunity to explore consistency over time in the RS and quantitative single gene levels (ER, PR, HER2).

Methods: All tumors successfully analyzed in the Genomic Health Laboratory from 1/1/05-3/31/11 were included. Descriptive statistics for the RS, the average reference gene expression level, and expression levels for quantitative single genes were obtained for each calendar year. This was done for the entire data set as well as for subgroups defined by histological tumor type. The associations by year between HER2 and GRB7, ER and HER2, and ER and PR expression levels were explored including scatterplots and summary statistics.

Results: There were a total of 207,691 breast cancer cases, and the number in each calendar year increased over time as shown in the Table. In general the median age increased slightly over time, as did the proportion of patients in the low RS Risk Group. There was no systematic change over time in the average reference gene expression level, or in the expression levels for the individual genes ER, PR or HER2 during the period from 2008 to 2011 when individual gene testing was provided as part of the Oncotype DX assay report. The relationships between HER2 and GRB7, ER and HER2, and ER and PR remained consistent over time.

Conclusions: Active monitoring of the Oncotype DX assay as mandated by ASCO/CAP shows a high degree of consistency in results for both the multigene Recurrence Score and the quantitative single gene results. These results and the approaches used for monitoring consistency are relevant to other institutions in their efforts to maintain and improve assay quality control.

P1-07-12
Assessment of Real World HER2 Status by Immunohistochemistry (IHC) and Fluorescence In Situ Hybridization (FISH) in Breast Cancers: Comparison with HERMark®, a Validated Quantitative Measure of HER2 Protein Expression.

Huang W, Paquet A, Sivaraman S, Pesano R, Goodman L, Sherwood T, Lie Y, Hickey J, Walworth C, Haddad M, Anderson S, Bates M, Weidler J. Monogram Biosciences Inc., South San Francisco, CA; Incyte Corporation, Wilmington, DE; Quest Diagnostics Nichols Institute, San Juan Capistrano, CA; Affymetrix, Santa Clara, CA; Gilead Sciences, Inc, Foster City, CA; Cepheid, Sunnyvale, CA

Background: Accurate assessment of the HER2 status is critical in determining appropriate therapy for patients with invasive breast cancer. ASCO/CAP HER2 testing guidelines caution that up to 20% of routine HER2 testing by IHC/FISH may be unreliable (Wolff et al. JCO 2007:25:118). The HERMark assay is a novel quantitative HER2 protein measurement for determining HER2 status in breast cancer. Central HER2 testing showed high concordance (96-98%) with HERMark for positive and negative categories when equivocal subsets were excluded (Huang et al. Am J Clin Pathol 2010; 134:303; Joesuus et al. 2008 SABCS, abstract 2071). In this study, we examined concordance between HERMark and routine HER2 testing by IHC and FISH from “real world” formalin-fixed, paraffin-embedded (FFPE) breast cancers submitted commercially for HERMark testing.

Methods: 717 HERMark results on FFPE breast cancers tested from 2008 to 2010 and corresponding HER2 IHC/FISH results were reviewed. The IHC and FISH results, per pathology reports submitted at the time of HERMark testing, were compared to HERMark categorical (negative, equivocal, positive) results.

Results: 590 (419) samples had IHC (FISH) and HERMark results available. Of these cases, 92% (94%) were either negative or equivocal by IHC (FISH). The HERMark testing reported 33% HERMark positive, 33% HERMark equivocal and 34% HERMark negative. Comparisons of HER2 status by IHC and FISH vs. HERMark are detailed in Table 1.

Table 1: Comparison of HERMark with HER2 IHC and FISH.

<table>
<thead>
<tr>
<th>HERMark</th>
<th>HER2 IHC</th>
<th>HER2 FISH</th>
<th>HER2 IHC</th>
<th>HER2 FISH</th>
<th>HERMark</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>413 (60%)</td>
<td>409 (58%)</td>
<td>14 (2%)</td>
<td>12 (2%)</td>
<td>2 (0%)</td>
</tr>
<tr>
<td>Equivocal</td>
<td>109 (16%)</td>
<td>111 (37%)</td>
<td>30 (21%)</td>
<td>25 (17%)</td>
<td>20 (14%)</td>
</tr>
<tr>
<td>Positive</td>
<td>59 (9%)</td>
<td>38 (6%)</td>
<td>10 (13%)</td>
<td>10 (15%)</td>
<td>30 (19%)</td>
</tr>
<tr>
<td>Total</td>
<td>581</td>
<td>568</td>
<td>44</td>
<td>42</td>
<td>35</td>
</tr>
</tbody>
</table>

Conclusions: Active monitoring of the Oncotype DX assay as mandated by ASCO/CAP shows a high degree of consistency in results for both the multigene Recurrence Score and the quantitative single gene results. These results and the approaches used for monitoring consistency are relevant to other institutions in their efforts to maintain and improve assay quality control.
Central HER2 FISH retesting of these samples will be performed and compared with HERmark results to further evaluate the discordance observed in this analysis.

P1-07-13  
Efficiency of a Laboratory Developed HER2 FISH Test on Circulating Tumor Cells.  
Mayer JA, Pham T, Wong KL, Scoggin J, Sales EV, Clarin T, Pircher TJ, Mikolajczyk SD, Cotter PD, Bischoff FZ. Biocept Inc, San Diego, CA

INTRODUCTION: Most circulating tumor cell (CTC) platforms rely on EpCAM for capture and cytokeratin (CK) for detection. However, an important population of cells that are CK-negative (i.e. cells with epithelial-mesenchymal transition (EMT) phenotype) will be missed. We report a new strategy to efficiently isolate a more heterogeneous population of CTCs using an antibody cocktail.

METHODS: In the first prospective study, blood (20 mL) was collected from 89 patients diagnosed with various late stage metastatic/recurrent cancers (breast, CRC, lung, prostate) following IRB approval. PBMCs were incubated with either EpCAM alone or a mixture of 10 capture antibodies to target both epithelial and mesenchymal cells. CTCs were subsequently captured in the OncoCEE™ channels and detected with cytokeratin (CK) and CD45. A second prospective IRB approved study involving 54 patients diagnosed with late stage metastatic/recurrent breast cancer was performed using similar detection strategies (CK cocktail mixture and anti-CD45) with the addition of HER2 FISH to determine amplification status among captured CK+/CD45- and CK-/CD45- cells.

RESULTS: In the first study, overall detection of CK+ cells was 83% with EpCAM alone and 93% with antibody cocktail. In addition, a median of 0.4 CK+ cells/mL and 1.0 CK+ cells/mL was observed using EpCAM and antibody cocktail, respectively. In the second study, CK+/CD45- cells were detected in 43 of 54 cases (80%). Among the 43 cases in which CK+/CD45- cells were detected, high concordance (93%) in HER2 status between primary tumor and CTCs was observed with HER2 amplification noted in both CK+/CD45- (50%) and CK-/CD45- (50%) cells.

CONCLUSIONS: We have developed a novel and robust method for CTC enumeration that utilizes a cocktail of antibodies for the detection of a heterogeneous (CK+ and CK-) population of CTCs. Our findings suggest an important population of CK- cells is being missed by current stain criteria in breast cancer patients. Data also demonstrate that recovery of CTCs from peripheral blood using the OncoCEE™ platform is efficient and suitable for FISH-based laboratory testing.

P1-07-14  
Quantum Dot-Labelled Antibodies To Assess HER2 Expression In Breast Cancer.  
Zona S, Blackburn E, Hojijaleslami AS, Brown IR, Gullick WJ. University of Kent, Canterbury, Kent, United Kingdom

Background: The Human Epidermal Growth Factor Receptor 2 (HER2) is overexpressed in 18-20% of breast cancers. Herceptin is an effective drug for the treatment of breast cancers expressing high levels of HER2. It is well known that there is a correlation between levels of HER2 expression and response to Herceptin. However a proportion of patients selected for Herceptin treatment do not respond to the drug. The accuracy of the assessment of HER2 levels in breast cancer is therefore important to predict patients’ response to Herceptin therapy. The current techniques in clinical use for the assessment of HER2 are immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH); however both IHC and FISH have several limitations which may result in patient misclassification. A more accurate technique, which can quantify HER2 levels more precisely, could help to improve clinical diagnosis, prediction of prognosis and allow more accurate select individual patients for particular drug therapies.

Materials and Methods: We employed QDs labelled antibodies, laser scanning confocal microscopy and image segmentation techniques to quantify HER2 expression in cases of formalin fixed paraffin embedded breast cancers. Quantum dots (QDs) are a new class of fluorochromes made of semiconductor nanocrystal which have several useful properties for quantitative image analysis. In order to validate the system we used a breast cancer HER2-IHC control tissue array with negative, low, moderate and strong expressing cases, and a tissue microarray containing 60 samples of formalin fixed paraffin embedded breast cancer sections, previously examined by IHC and scored semi-quantitatively (0-3+). We then applied this system to quantify HER2 expression a tissue microarray containing 150 primary breast cancers scored as 3+ by IHC from patients treated with Herceptin.

Results: We first demonstrated that the QD system could reliably detect HER2 expression in IHC 3+ cases. A comparison of immunofluorescent staining with conventional immunohistochemistry showed that QDs give more linear and scalable measurements of receptor levels. We also quantified HER2 within a set of 150 breast cancers scored as 3+ by IHC, and we found that HER2 is expressed at very different levels, ranging over fifty fold between individual (IHC3+) cases. We currently are evaluating the correlation between HER2 receptor levels, measured by QDs, and patient’s response to Herceptin.

Conclusion: QDs and image analysis can produce a more precise measurement of HER2 levels of expression than IHC. This should help to improve clinical diagnosis, prognosis, identify specific treatments for individual patients and reduce costs associated with Herceptin treatment. This technology may be applied to study other members of the EGF family in breast cancers or more widely as a quantitative measurement of biomarkers in tumours.

P1-07-15  
The Reliability of Ki-67 Expression Assessment Using Core Needle Biopsy and Surgical Specimens of Invasive Breast Cancer: Can Ki-67 Change Predict Benefits of Preoperative Endocrine Therapy?  
Mizuno Y, Takayanagi H, Sato K. Tokyo-West Tokushukai Hospital, Akishima-City, Tokyo, Japan

Background: The use of Ki-67 change as a predictive marker in breast cancer has been widely investigated in patients treated with preoperative endocrine therapy. It has been assumed that Ki-67 expression determined using core needle biopsy and surgical specimens of invasive breast cancer is concordant. Many studies have suggested the concordance of the results obtained using core needle biopsy and surgical specimens for expression of the estrogen receptor (ER), progesterone receptor (PgR), and human epidermal growth factor receptor type2 (HER2) status in early-stage breast cancer. However, there was few study to compare Ki-67 expression in the core needle biopsy and surgical specimens. We conducted a retrospective study of Ki-67 expression assessment between the two types of specimens to answer this question.

Methods: A total of 269 patients underwent primary operations for early-stage breast cancer at Tokyo-West Tokushukai Hospital from August 2008 to May 2011. Among these patients, 169 patients who received core needle biopsy prior to operation were enrolled.
Patients with ductal carcinoma in situ and those with neoadjuvant chemotherapy were excluded. A cutoff value of 20% was used for Ki-67-positive criteria. We also examined ER, PgR, and HER2 expression and compared it with Ki-67 expression. Statistical significance for concordance rates between the two types of specimens was evaluated by Wilcoxon t-test. To evaluate the consequence of formalin and genetic heterogeneity, parameters such as operative method and tumor size were analyzed by χ² analysis.

**Results:** The concordance rate between the two types of specimens for Ki-67 expression was 76%, and this was significantly lower than that for ER expression, which was 96%. The concordance rates for PgR and HER2 expression were 88% and 91%, respectively, and they were not significantly different from the rate for Ki-67 expression. 45 patients (34.6%) had received mastectomy in the Ki-67 concordant group, and 14 patients (34.1%) in the discordant group. 55 patients (42.0%) showed T2 tumor size in the concordant group, 13 patients (31.7%) in the discordant group. No significant differences of parameters such as operative method (mastectomy vs breast conserving surgery) and tumor size (T1 vs T2) were observed between the two patient groups.

**Conclusion:** The reliability of Ki-67 concordance rate assessment using core needle biopsy specimens was significantly lower than that of ER expression. A standard pathological assessment of Ki-67 expression might be needed for the possible use of Ki-67 as a predictive marker for preoperative endocrine therapy.

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**P1-07-16**

**Number Needed To Count: A Novel Model for Ki67 Assessment in Breast Cancer.**

**Background:** Tumor proliferation assessed by Ki67 is frequently used as a prognostic and above all treatment predictive marker in breast cancer where a decrease in Ki67 expression, following neo-adjuvant therapy, may be interpreted as a reliable treatment effect. Previous studies have indicated that the optimal number of tumor cells needed to count may vary from case to case. The standard error of the estimated proportion will decrease with increasing number of tumor cells counted (n), but a dilution bias will affect the estimate in cases with few tumor cells within a hotspot area. Hence, a large n is appropriate in Ki67-homogeneous hotspot areas and inappropriate, at least for small hotspots, in case of heterogeneity. Further, a chosen cut-off value has implications for the choice of n. A low n may be sufficient for extreme proportions, but the closer the true unknown proportion is to the cut-off, the larger n will be required. The lack of consensus concerning Ki67 assessment may jeopardize the comparison of research results. Hence, a standardized counting model is warranted.

**Material and Methods:**

Exact two-sided confidence intervals for proportions based on the binomial distribution were used to derive rejection regions for sequential testing of the null hypothesis that the fraction of Ki67-positive cells is equal to the chosen cut-off (20% in this study). A lower limit of 50 counted tumor cells to get a reasonably stable estimate and an upper limit of 400 tumor cells to prevent extreme dilution bias for small hotspots, were applied. Briefly, the counting strategy can be explained as follows: Locate a hotspot and count 50 tumor cells. If the Ki67 estimate belongs to the upper or lower rejection region, stop counting. If not, count another 10 tumor cells and perform a new hypothesis test. Proceed until either the null hypothesis has been rejected or the upper limit of 400 has been reached. Simulation was used to determine that a nominal significance level of α=0.01 for each test will keep the overall probability of falsely rejecting the null hypothesis fixed at 0.05. The novel counting strategy was compared to static counting of 200 tumor cells using 100 Ki67-stained breast cancer samples.

**Results:** The median number of tumor cells needed to count to determine Ki67-status was 100 and the average 175. The rejection region was reached immediately after 50 tumor cells counted for 32 samples with Ki67-levels far from the cut-off, whereas counting 400 tumor cells was insufficient for classification in another 18 samples. In samples classified as highly proliferative (>20%), the mean Ki67-estimate was 49% using the counting model compared to 42% using a fixed denominator of 200 tumor cells. The largest absolute difference between the two estimates for these 32 samples was 23% - from 42% (model) to 19% (static), thus more than a factor two and a dilution effect leading to changed Ki67-status.

**Conclusions:** Estimation of the fraction of Ki67-positive tumor cells using a fixed denominator may be inadequate – especially for small hotspots. We hereby propose a strategy for tumor cell count optimization that hopefully will contribute to standardization of the counting practice for tumor proliferation assessed by Ki67.

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**P1-07-17**

**Multiplex Plasma Biomarkers Associated with Bone Metastasis from Breast Cancer.**

**Background:** Over two-thirds of breast cancer patients who relapse will develop bone metastasis, and there are currently no effective biomarkers to predict for this event. Multiplex immunoassay technology for 60 bone metastasis-associated biomarkers was conducted on pretreatment plasma from 40 patients enrolled in a 2nd-line phase III hormone therapy trial of metastatic breast cancer, to discover effective circulating biomarkers predictive of bone metastasis.

**Methods:** Pretreatment EDTA plasma from 20 patients with bone metastasis, and 20 patients with non-bone metastasis from the letrozole-megace 2nd-line phase III hormone therapy trial of metastatic breast cancer was evaluated using Searchlight multiplex immunoassay technology (Aushon Biosciences, Billerica, MA) on 60 biomarkers custom-selected for known association with bone metastasis. Multiplex data analysis for maximal discrimination of bone metastasis vs. no bone metastasis cohorts was performed by two independent statisticians: one evaluated the predictive capacity of individual biomarkers as well as two-marker combinations constructed by Monte Carlo optimization using Metropolis algorithm (MMC), and the other used a machine learning technique called hybrid bootstrapped RLDA + PCA (regularized linear discriminant analysis with principal component analysis, with dimensionality reduction used in the bootstrapping procedure).

**Results:** One of the 60 analytes (IL-17) was undetectable at the 1.6 pg/ml lower limit of detection, and was not included in the multiplex statistical analyses. Of the remaining 59 analytes, MMC multiplex analysis yielded 9 biomarkers that predicted for bone metastasis, whereas RLDA + PCA multiplex analysis yielded 20 predictive biomarkers. Common to both analyses, which were limited in this
small 40 patient study, were 5 biomarkers, with varying functions including growth factors, cytokines, inflammatory factors, protease inhibitors, and cell adhesion molecules.

Conclusions: Multiplex analysis of 60 biomarkers custom-selected for known association with breast cancer metastasis to bone yielded 5 pretreatment plasma biomarkers classified as predictive of bone metastasis by 2 different multiplex statistical analysis methods. Evaluation of these biomarkers is now warranted in a larger validation cohort of breast cancer patients with and without bone metastasis.

P1-07-18
Expanding an Online Tool for Genome-Wide Validation of Survival-Associated Biomarkers in Breast and Ovarian Cancer Using Microarray Data of 3,862 Patients.

Gyorffy B, Laneczky A, Szallasi Z. Semmelweis University, Budapest, Hungary; Harvard Medical School, Boston

The pre-clinical validation of prognostic gene candidates in large independent patient cohorts is a pre-requisite for the development of robust biomarkers. We earlier implemented an online tool to assess the prognostic or predictive value of the expression levels of all microarray quantified genes in breast cancer patients. In present study, we further expanded our database, added additional analytical options and implemented the tool for ovarian cancer patients. The database was set up using gene expression data and survival information of breast and ovarian cancer patients downloaded from GEO and TCGA (Affymetrix HGU133A, HGU133A 2.0 and HGU133+2 microarrays). After quality control and normalization only probes present on all three Affymetrix platforms were retained (n=22,277). Patients can be stratified into the various robust subtypes either by histology or by various gene expression profiling based methods. To analyze the prognostic value of the selected gene in the various cohorts the patients are divided into two groups according to the median expression of the gene. A Kaplan-Meier survival plot is generated and significance is computed. All together 2,472 breast cancer patients and 1,390 ovarian cancer patients were entered into the database. These groups can be compared using relapse free survival (n=2,414 in breast cancer and 1,090 in ovarian cancer), or overall survival (n=463 and n=1,290). Follow-up threshold has been implemented to exclude long-term effects. The combination of several probe sets can be employed to assess the mean of their expression as a multigene predictor of survival and therapy efficiency. In summary, we expanded our global online biomarker validation platform to mine all available microarray data to assess the prognostic power of 22,277 genes in 2,472 breast and 1,390 ovarian cancer patients. The tool can be accessed online at: www.kmplot.com/breast and www.kmplot.com/dev/ovar.

P1-07-19
Analysis of HER2-Status in Breast Cancer by Mass Spectrometry in Archival, Formalin-Fixed Tissues.

Sanders M, Sprung R, Ham A, Sanchez V, Manning S, Arteaga C, Liebler D. Vanderbilt University Medical Center, Nashville, TN

HER2 (ERBB2) is overexpressed in about 25% of breast cancers and predicts clinical benefit from trastuzumab, as well as response to anthracycline-based chemotherapy. Fluorescence in situ hybridization (FISH) to detect HER2 gene copy number and immunohistochemistry (IHC) to detect HER2 protein levels are approved by the FDA to identify HER2-positive (H2) tumors. However, the 2007 ASCO/CAP report concluded that approximately 20% of HER2 testing may be inaccurate. Further, the available data did not clearly demonstrate clear superiority of either IHC or FISH as a predictor of benefit from anti-HER2 therapy. Discordance between these methods is as high as 5%. Thus, novel complementary quantitative methods for interrogating HER2 expression in tumors are needed.

Targeted protein analysis by multiple reaction monitoring mass spectrometry (MRM-MS) offers a powerful approach to configure assays for specific proteins without using antibodies. Our studies using this platform have demonstrated applicability to formalin-fixed, paraffin-embedded (FFPE) specimens. In the current studies, we used this approach to measure signals from two tryptic peptides specific to HER2, one each from the extracellular and intracellular domains, selected from among 28 candidates based on their signal intensity and sharpness of their chromatographic profiles. Preliminary studies with a HER2-overexpressing BT474 xenograft in mice demonstrated quantitation and detected previously reported HER2 ectodomain shedding. Subsequent analysis of FFPE tissue from five H2 and five triple-negative (TN) tumors yielded measurement of at least 1 femtomole of receptor for H2 tumors and less than 0.2 femtomole of receptor for TN tumors per microgram of digest analyzed. If we assume 200 picograms of protein per cell, the results suggest 110.00 to 468,000 receptors per cell in the H2 tumors and only 2,000 to 14,000 receptors per cell in the TN tumors. Despite significant biological variability in receptor levels measured among the specimens of each type, a clear separation of the H2 and TN tumors was achieved based on the peptide quantitation. This preliminary study demonstrates the potential of MRM-MS in FFPE tissue to provide an alternate approach to IHC-based protein analysis. MRM-MS offers the potential for more, accurate and robust HER2 quantification in clinical breast cancer tissues. The next phase of this work will encompass a larger sample set, including tumors with equivocal and negative FISH and/or IHC test results. Correlation with response to anti-Her2 therapy will be performed in samples with available follow-up data.

P1-07-20
Consistent High False Negative Rate of HER2 qRT-PCR of Oncotype DX® in Comparison to ASCO/CAP Recommended Combined IHC/FISH Method.

Dubbs DJ, Bhargava R. Magee-Womens Hospital of UPMC, Pittsburgh, PA

Background

In our prior multi-institutional study of 843 cases we have shown a high false negative rate for HER2 testing by the oncoTYPE DX® RT-PCR method, (ODX) compared to our combined FISH and IHC approach [J Clin Oncol 29: 2011 (suppl; abstr 603)]. This study is part of an ongoing quality assurance study, comparing HER2 test results by FISH/IHC and the ODX.

Methods

From July 2010 till April, 2011, all patients who had either positive or equivocal HER2 results (on core biopsy) at Magee-Womens Hospital (MWH) by combined IHC/FISH, and who also had the ODX ordered (on resection specimen) by an oncologist, were reviewed for positive concordance of HER2 results. All tissues were fixed and FISH/IHC was reported according to ASCO/CAP HER2 guidelines. The core biopsy HER2 positive results were also confirmed positive on the same tissue block that was tested by ODX.

Results

Of the total 234 cases, there were 21 cases that were either classified as positive (n=8) or equivocal (n=13) by combined IHC/FISH approach at MWH. Of the 8 MWH+ cases, 4 showed 3+ IHC score and these were also positive by ODX. The remainder 4 MWH+ cases were IHC 2+, and unequivocally amplified by FISH. These 4 cases were
either equivocal (2 cases) or negative (2 cases) by ODX. Thirteen cases reported as equivocal by MWH FISH, were all reported as negative by ODX. Of the 213 MWH negative cases, 212 were also negative by ODX (percent negative agreement of >99%) and one case was called equivocal. If all equivocal cases are excluded, then percent positive agreement (PPA) between IHC/FISH and ODX is 67% (4/6). If “equivocal” cases are considered as “negative”, then PPA is 50% (4/8). If “equivocal” cases are considered as “positive”, then PPA is only 29% (6/21).

Conclusions
(1) Similar to our prior study [J Clin Oncol 29: 2011 (suppl; abstr 603)], there is a consistent poor percent positive agreement for HER2 testing between FDA cleared FISH/IHC methods and ODX, and these results cast serious doubt on the robustness/reliability of ODX results for HER2. (2) There should be serious concern raised over the reliability of the other multiplex IHC product results of ODX, and likely, the ODX recurrence score for an individual patient, as this sensitivity data suggests inadequate tissue microdissection, or issues with the ODX test platform. (3) Clinicians need to be aware that HER2 results of ODX are not reliable for patients who are equivocal or amplified by FDA approved methods, and only FDA approved methods should guide treatment decisions.

P1-07-21
Analysis of Molecular Markers by Immunohistochemistry (IHC) Method on Formalin Fixed Paraffin Embedded (FFPE) Tissues Could Predict Shorter Recurrence Free Survival (RFS) and Overall Survival (OS) among Patients Who Have Received Adjuvant Chemotherapy for Early Breast Cancer.
Moe M, Gee J, Finlay P, Mansel R, Adams R, Singleton Hospital, Swansea, United Kingdom; Velindre Hospital and Cardiff University, Cardiff, United Kingdom; Cardiff University, Cardiff, United Kingdom

Background: Various molecular markers assessed by IHC (ER, PR, HER2) and gene expression profiling (e.g. Oncotype Dx) have been developed as prognostic and predictive tools for breast cancer. Gene profiling is said to be superior to IHC but at a considerable cost with limited availability. IHC is relatively inexpensive and more readily available. If early breast cancer patients who are going to relapse within 5 years of curative surgery despite adjuvant chemotherapy could be identified by IHC on FFPE tissue alternative adjuvant therapies could be explored. In this context, we here evaluate IHC for expression of a panel of molecular markers implicated in: growth signalling pathways (ER, PR, HER2, EGFR, CD71, Ki67, MCM2), cell survival (Bcl-2, Bag 1), angiogenesis (PDGFrA) and cell cycle progression (Aurora A, MCM2). Of note, this study includes markers of breast cancer molecular subtype (ER, PR, HER2, Ki67, EGFR, also CK5/6) and several proteins encoded by genes in the Oncotype Dx test (ER, PR, HER2, Ki67, Bcl2, Bag1 and CD68).

Materials and Method: 72 cases (R) relapsing within 5 years of curative surgery, 72 controls (C), relapse free > 5 years were identified from the hospital records. All patients had adjuvant chemotherapy. Controls were matched to cases by Adjuvant! recurrence risk (ARR). Optimised IHC was performed on FFPE TMA slides using a Ventana autostainer. Protein expression was evaluated on digitalised images (Mirax scanner). Survival analysis by molecular markers expression and also 5 molecular subtypes, Luminal A (LA = ER/PR+, HER2−, Ki67−), Luminal B (LB = ER/PR+, HER2/Ki67+), HER2 enriched (H = ER−, PR−, HER2+), Core Basal (CB = ER−, PR−, HER2−, CK5/6/EGFR−) and 5-negative (5N = negative for ER, PR, HER2, EGFR, CK5/6), were performed. SPSS 16v. was used for statistical analysis.

Findings: All but four cases had died at the time of analysis. Four controls developed relapse at 83.8, 90.6, 107.7, 127.6 months respectively. Two controls died from non-breast cancer causes. Median follow-up for the controls group (ie. mOS) was 104.9 mo (72.8 - 164.4). For cases, mRFS and mOS were 23.2 (4.5 - 59.9) and 39.7 (8.1 - 129.7). mRFS and mOS for HER2 molecular subtypes were: Subtype LA = not yet & not yet; LB = 58.1 & 86.1; CB = 15.4 & 30.4; H = 28 & 55.9; 5N = 19.9 & 26 (p < 0.0001 & <0.0001 by Log rank test). Better RFS and OS were found for positive Bcl2 (p = 0.036 & 0.058) and MCM2 (p = 0.022 & 0.048), negative Aurora A (p = 0.01 & 0.001) and PDGFRA (p = 0.07 & 0.086) expressions. For this study cohort there was no correlation between ARR and survival outcome or molecular subtypes. Result of ongoing multivariate analysis and correlation between survival and CD68, CD71 and Bag 1 expressions will be presented in the conference.

Discussion: Subtypes CB & 5N, negative Bcl-2 & MCM2, positive Aurora A & PDGFRA expression as measured by IHC were predictive of poor RFS and OS. While these findings need to be verified in an independent cohort, IHC profiles nevertheless have potential to stratify different risk groups for clinical trials and effective adjuvant treatments.

P1-07-22
A Venezuelan Study of Breast Cancer Estrogen Receptor, Progesterone Receptor and HER2 Receptor Expression by the Standard Method, Immunohistochemistry (IHC), Compared to a New Method, Quantitative Reverse Transcription Polymerase Chain Reaction (RT-PCR).
Marin C-EM, Ramirez AC, Baehner FL, Yoshizawa C, Acosta MM. Fundacion BADAN; Caracas, Miranda, Venezuela; Genomic Health, Redwood City, CA

Background: Assessment of ER, PR and HER2 is of significant importance in breast cancer diagnosis and treatment. We have performed the first large assessment of central IHC for ER, PR and HER2 by a South American (Caracas, Venezuela) Central Lab to Central Lab RT-PCR by Oncotype DX®.

Design: Breast cancer specimens from the Fundación BADAN were evaluated by IHC for ER (clone 1D5), PR (clone 636) using 1% staining for positivity using ASCO/CAP guidelines and HER2 (clone A0485) using ASCO/CAP guidelines of 3+ in ≥30%. Standardized quantitative RT-PCR analysis for ER and PR used Oncotype DX with the pre-defined cutoffs of 6.5 units and 5.5 units for positivity, respectively. For HER2 the standard pre-defined cutoffs were used: positive ≥11.5 units, equivocal >10.7–<11.5 units, and negative ≤10.7 units. For HER2 concordance analysis the equivocal range was excluded from both assays according to ASCO/CAP Guidelines (Wolff et al, 2006). For all quantitative RT-PCR assessments the reference normalized expression measurements ranged from 0 to 15, where each 1-unit increase reflects about a 2-fold increase in RNA.

Result: Evaluable data was obtained in 96 pts for ER, 95 pts for PR (1 patient assessment unavailable) and 89 pts for HER2 (7 patient assessments unavailable). Two-by-two tables (below) compare IHC versus RT-PCR for ER, PR and HER2. The overall concordance for ER between IHC and RT-PCR was 94% (Kappa 0.467; 95% CI 0.307, 0.628); for PR between IHC and RT-PCR was 92% (Kappa 0.668; 95% CI 0.457, 0.878); and for HER2 between IHC and RT-PCR was 97% (Kappa = 0.649; 95% CI 0.280, 1.000). Three IHC 2+ cases and 4 equivocal cases by Oncotype DX were excluded from Concordance and Kappa statistics.

Conclusion: RT-PCR by Oncotype DX for ER, PR and HER2 status is useful for active monitoring of IHC assays as mandated by ASCO/
CAP guidelines. There is a high degree of overall concordance between central IHC performed in South America (Caracas, Venezuela) and central RT-PCR for ER, PR and HER2 status.

### Table: Concordance for IHC versus RT-PCR for ER, PR and HER2

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HER2 Excludes Equivocal Cases per ASCO/CAP guidelines

### P1-07-23

**Absolute Quantification of Estrogen Receptor alpha in Breast Cancer.**

Britton D, Scott G, Russell C, Held J, Ward M, Benz C, Pike I. Proteome Sciences Plc, London, United Kingdom; Buck Institute, Novato, CA

Estrogen receptor alpha (ER) is the first biomarker to have been clinically validated as a predictor of cancer therapy response. Measurements of tumor ER expression were based on radiolabeled ligand binding to receptor present in tumor lysates. Despite substantial tissue requirement, lack of specificity for ER isoforms (alpha versus beta) or receptor integrity, quantitative determination of tumor ER content (fmol/mg total protein) was possible. Introduction of anti-ER antibodies later permitted immunohistochemical (IHC) evaluation of ER expression. IHC determination of ER status in newly diagnosed breast cancer is now a standard of care. While these IHC assays have been shown to be as predictive of endocrine responsiveness as ligand binding assays, they remain semi-quantitative at best reporting tumor ER status either categorically (e.g. + or -) or as a numeric score which is subjective and lacks a linear relationship with endocrine responsiveness. Thus the lack of precision for quantifying ER as a predictive biomarker is one of the most important unresolved issues in breast cancer. We are working to develop a proteomic liquid chromatography-mass spectrometry (LC-MS) assay to help resolve this issue. Samples included recombinant ER (rER), immunoprecipitated (IP) rER, and IP ER from MCF7 cells. ER was digested with trypsin, lyophilised and solubilised in 5 femto-mol/ microliter (100 μl) heavy peptide internal standard mix. ER peptides were resolved by LC (100 μl/minute) and detected by selected reaction monitoring MS. The area under the total ion chromatogram for each peptide was used to quantify the amount of analyte present in each sample as a single point reference to the signal of the heavy peptide spike. An 11 point calibration curve (0.1-1000 fmol on column (o/c)) of light peptides with each point in the curve spiked with 100 fmol heavy peptide was also produced to determine assay characteristics such as limits of detection (LOD), limits of quantification (LOQ), linearity, accuracy and precision. Three ER peptides were selected for quantification as they gave the greatest LOD, LOQ, linearity as well as reasonable intra- and inter-assay precision following multiple digestions of rER (intra = 3 digestions in 1 day; inter = 9 digestions over 3 weeks).

Following IP of ER from four replicates MCF7 cell lysates (1mg/ ml total protein) and measurement of ion intensities of the three ER peptides the mean concentration of ER was calculated to be 52 fmol (S.D of 7.5 fmol; n=4) per mg of total cell lysate after normalising for IP efficiency. We continue to develop the method to improve sensitivity and normalise for variability in IP and digestion. With the inclusion of reference peptides to known ER phosphorylation sites we are also in the process of quantifying ER phosphorylation. We aim to accurately determine ER concentration and phosphorylation status in tumor lysates and assess how these correlate with responsiveness to antiestrogen therapies.

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**P1-07-24**

**Quantitative and Immunohistochemical Detection of Breast Cancer Cells in Blood Samples.**

Andergassen U, Zebisch M, Kölbl AC, Schindelbeck C, Jäger B, Hepp P, Janni W, Jeschke U, Friese K, Rack BJ. Ludwig-Maximilians-University Muenchen, Munich, Bayern, Germany; Heinrich-Heine-University Düsseldorf; Duesseldorf, NRW, Germany; Klinikum Traunstein, Traunstein, Bayern, Germany

**Background**

Disseminated tumor cells (DTCs) in bone marrow and circulating tumor cells (CTCs) in blood are found in patients with epithelial carcinomas (breast cancer) and are often correlated with poor prognosis of the disease. In current models circulating tumor cells (CTCs) dissolved from the primary tumor are thought to be responsible for the occurrence of metastases. However, the detection of CTCs is still a technical challenge. In this study, two methods for tumor cell detection of patients’ samples are presented (Real-Time-PCR and immunohisto-chemical staining). Both are simple and known methods with a high sensitivity and a spread marker panel.

**Materials and Methods**

For the implementation of both methods, different breast cancer cell lines have been used (Cama-1, MCF-7; ZR-75-1). For Real-Time-PCR, blood samples of a healthy donors were spiked with different cell counts (0, 10, 100, 1000, 10.000 and 100.000) per ml blood sample. Total RNA was isolated from the samples, reversely transcribed into cDNA and used for TaqMan Real-Time-PCR reaction with probes against CK8, 18 and 19, while 18s was used as reference. Relative Quantification Curves were drawn by Microsoft® Excel®. For immunohistochemical staining, cytospins were prepared from spiked blood samples, fixed with acetone, air dried and stained with antibodies against Her2- and Thomsen-Friedenreich-Antigen (CD176). In a second staining step fluorescently labelled secondary antibodies were applied. Nuclei were counterstained with DAPI, TF-Antigen was stained by Cy2 and Her2-Antigen by Cy3. The staining was controlled and documented by an epifluorescence microscope.

**Results**

The curve of Relative Quantification for MCF-7 and ZR-75-1 cells shows an increasing slope starting from 1000 cells. For the Cama-1 cell line this trend is already seen from 10-100 cells. In ZR-75-1 all three genes analysed reveal this trend, whereas in Cama-1 and MCF-7 cells a strong increase in Relative Quantification is especially seen for CK8 and 18. In the immunohistochemical staining, the cells were considered as tumour cells if they showed staining with the antibody-combinations used. Stained cells were counted and recovery rates were determined. For ZR-75-1, 17 of 30 cells which were spiked in the blood samples were recovered. For MCF-7, 18 cells were found in average, and for Cama-1 23 cells were located per slide. The recovery rates calculated from these numbers are 56,6% and 60,0% for ZR-75-1 and MCF-7, for Cama-1 the recovery rate reaches 76,6%. Conclusion It seems that Cama-1 cells are a better model than MCF-7 and ZR-75-1 for Real-Time PCR quantification of mamma carcinoma.
tumor cells in blood samples. MCF-7 and ZR-75-1 cells tend to react more likely immunologically with blood cells of the donor (agglutination between blood cells and cancer cells). The Cama-1 cell line shows also advantages in the detection of tumor cells using immunohistochemical staining. Therefore it will be necessary to test both methods on patient samples to proof their benefit.

**PI-08-01**

**Survival in Metastatic Breast Cancer (MBC): No Evidence for Improved Survival Following Distant Recurrence after Adjuvant Chemotherapy.**

Tevaarwerk AJ, Gray R, Schneider BP, Smith ML, Wagner LI, Miller KD, Sparano JA. University of Wisconsin-Carbone Cancer Center; Indiana University-Simon Cancer Center; Northwestern University, Chicago, IL; Dana-Farber Cancer Institute; Research Advocacy Network; Albert Einstein University-Montefiore Medical Center

Population-based studies have suggested improved survival for patients diagnosed with MBC in recent years, presumably due to the availability of new and more effective therapies (Chia et al. Cancer 2007; Dawood et al. JCO, 2008). The objective of this analysis was to determine if survival improved for patients who participated in Eastern Cooperative Oncology Group (ECOG) adjuvant trials and later developed MBC.

**Methods:** Adjuvant trials coordinated by the ECOG that accrued patients between 1978 and 2002 were reviewed (n=12), which included followup until 2010. Cytotoxic and biologic agents approved for MBC during this time included paclitaxel (1994), capetcitabine and trastuzumab (1998), docetaxel and gemcitabine (2004), lapatinib and ixabepilone (2007), and bevacizumab (2008). Survival following distant recurrence was estimated for 4 time periods ranging from 6-10 years, and adjusted for baseline covariates in a Cox proportional hazards model. Because distant relapse free interval (DRFI) was the covariate most strongly associated with survival after recurrence, and the potential for “gap time” bias this could introduce, logrank tests for other covariates and estimates of effects were computed stratified on DRFI (0-3, >3-6, > 6 years). HER2 status was not routinely available and thus not included.

**Results:** The 12 trials included 14,752 patients (93% received adjuvant chemotherapy); 3711 (25.2%) developed distant recurrence. Median survival after distant recurrence was 20 months; the estimated 5 and 10-year survival rates were 16.3% and 6.1%, respectively. Median survival by time period is shown in the table, stratified by DRFI. Median survival did not significantly change over time by DRFI (≤3 years, p=0.15; >3 yr, p=0.57). In a Cox proportional hazards model, factors associated with inferior survival after adjusting for other covariates included shorter DRFI (<3 years vs. 3-6 years - hazard ratio [HR] 1.60, p<0.001, and > 6 years vs. < 3 years - HR 2.23, p <0.001), ER-negative disease (HR 1.30, p<0.001), PR-negative disease (HR 1.36, P=0.0001), number of positive axillary nodes at diagnosis (1-3 vs. 0 nodes - HR 1.28, 4-9 vs. 0 nodes - HR 1.51, > 9 vs. 0 nodes - HR 1.51, p=0.0001), and black vs. white race (HR 1.29, p=0.0003), but not age at recurrence (p=0.15). When the year of recurrence was added to the Cox proportional hazards model using the intervals shown in the table below, it was not significantly associated with survival. Results were similar when 1978-2010 was assessed by 5-6 year intervals.

**Conclusions:** In contrast to reports from population-based studies, we do not observe any improvement in survival over time for patients who develop distant recurrence after adjuvant chemotherapy. There remains a critical unmet need for new therapies for MBC, especially for those who recur after adjuvant chemotherapy.

**PI-08-02**

**Pre-Diagnosis Body Mass Index and Breast Cancer Prognosis and Survival: Report from the after Breast Cancer Pooling Project.**

Kwan ML, Chen WY, Weltzien E, Beasley JM, Lu W, Nechuta SJ, Quesenberry CP, Pierce JP, Shu XO, Caan BJ. Kaiser Permanente Division of Research; Brigham and Women’s Hospital and Harvard Medical School; Fred Hutchinson Cancer Research Center; Shanghai Institute of Preventive Medicine; Vanderbilt University; University of California, San Diego

Background: A large body of evidence dating back over 30 years suggests that obese women have poorer survival after a breast cancer (BC) diagnosis compared to non-obese women. Despite most studies supporting an association of elevated risk of overall mortality with obesity, the relationship of obesity with risk of BC recurrence, BC mortality and non-BC mortality remains unclear. Furthermore, reports suggest that the association of BMI with BC outcomes may be U or J shaped, prompting the necessity of examining underweight and more severely obese women as independent groups. We conducted a pooled investigation of pre-diagnosis BMI and BC recurrence and survival using data from the After Breast Cancer Pooling Project (ABCPP).

Materials and Methods: The ABCPP includes 14,950 BC survivors from four prospective cohorts (three US and one Shanghai, China) diagnosed from 1990-2006 with invasive primary AJCC Stage I-III BC at ages 20-83 years. A random effects meta-analysis was conducted to assess heterogeneity across studies and poolability of data. Delayed entry Cox proportional hazards models were used to estimate hazard ratios (HR) and 95% confidence intervals (CI) for the associations of pre-diagnosis BMI (underweight <18.5 kg/m², normal 18.5-<25 kg/m², overweight 25-30 kg/m², obese ≥30 kg/m²) with BC recurrence, BC death, non-BC death, and overall death, adjusted for age at diagnosis, stage, race/ethnicity, menopausal status, hormone receptor status, number of positive lymph nodes, treatment, smoking history, and comorbidity (diabetes, hypertension, and/or CVD). Subgroup analyses further divided the obesity group into obese (30-<35 kg/m²), severely obese (35-<40 kg/m²), and morbidly obese (≥40 kg/m²) categories.

**Results:** No heterogeneity in effect estimates by study was found. 2104 deaths (1416 BC-related) and 2320 recurrences were observed after a mean (SD) of 7.66 (3.95) years of follow-up. Both underweight and obese women had a statistically significant increased risk of overall death compared to normal-weight women (underweight HR=1.69; 95% CI: 1.25, 2.28 and obese HR=1.74; 95% CI: 1.36, 2.22; p for non-linear association=0.01). Similar associations were found for non-BC death. Obese but not underweight was associated with increased risk of BC death (HR=1.17; 95% CI: 1.01, 1.36) and recurrence (HR=1.11; 95% CI: 0.98, 1.26). When examining finer obesity categories, the morbidly obese women had the greatest risk for all outcomes (overall death HR=1.90; 95% CI: 1.48, 2.45; non-BC death HR= 3.27; 95% CI: 2.25, 4.77; BC death HR = 1.47; 95% CI: 1.05, 2.06; recurrence HR = 1.27; 95% CI: 0.95, 1.71). No effect modification was observed by menopausal status, hormone receptor status, chemotherapy, and smoking. In all analyses, overweight women had similar risk of outcomes compared to normal-weight women.

**Discussion:** In this large pooling study of nearly 15,000 BC survivors, we found that the association between BMI and BC outcomes,
specifically overall death and non-BC death, was U shaped with both underweight and obese women at greatest risk. Morbidly obese women were at even greater risk compared to other obesity groups. Maintaining a healthy weight throughout adult life may be beneficial for BC prognosis and survival.

**P1-08-03**

**Huge Improvement in Relapse-Free Breast Cancer Survival over the Last 25 Years.**

Geurts SME, van Dijck JAAM, de Vegt F, Paquay Y, Siesling S, Verbeek ALM, Tjan-Heijnen VCG. Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands; Hospital Bernhoven, Oss, Netherlands; Comprehensive Cancer Centre, Netherlands; Maastricht University Medical Centre, Maastricht, Netherlands

**Introduction** The purpose of this study was to evaluate the dwindling risk of relapse in breast cancer patients treated with curative intent since the 1970s.

**Patients and methods** 8570 women diagnosed with invasive breast cancer in 2003 or 2004 were compared with 307 consecutively diagnosed patients with invasive breast cancer between 1972 and 1986 in the Netherlands. Five-year overall survival and 5-year risk of relapse, i.e., second primary breast cancer, locoregional recurrence or distant metastasis, were calculated using the Kaplan-Meier method. Multivariable Cox-proportional hazards modelling was applied to correct the period-specific risk of relapse for tumour size, lymph node involvement and age at diagnosis.

**Results** Median (range) age at diagnosis was 52 years (27-82 years) in 1972-1986 and 58 years (20-96 years) in 2003-2004. Patients diagnosed in 1972-1986 had a larger tumour size and similar nodal involvement as compared to patients diagnosed in 2003-2004. In 2003-2004 more women were treated with breast conserving surgery, chemotherapy, and hormonal therapy as compared to 1972-1986. The 5-year overall survival rate increased from 71% (95% CI: 65% - 76%) in the period 1972-1986 to 85% (95% CI: 84% - 85%) in the years 2003-2004. Five-year risk of any relapse decreased from 37% to 16% across the calendar years (table 1). This decrease was observed for the risk of locoregional recurrence and the risk of distant metastasis (table 1). Risk of second primary breast cancer was similar for both periods of diagnosis (table 1). After adjustment for tumour size, nodal status and age at diagnosis the decrease in risk of relapse remained significant for patients diagnosed in 2003-2004 compared to 1972-1986 (HR= 0.5, 95% CI: 0.4 - 0.6).

**Conclusion** Over the last decades, the risk of breast cancer relapse has tremendously decreased, also after adjustment for tumour stage and age at diagnosis. The improved prognosis can be explained by the more often administered and intensified systemic treatment procedures and, further, by an earlier detection. The similar nodal stage probably resulted from stage migration caused by a more thorough performed lymph node staging in 2003-2004 compared to 1972-1986.

**Summary of hazard ratios by BMI categories and treatment groups**

<table>
<thead>
<tr>
<th>BMI category</th>
<th>All patients</th>
<th>DFS</th>
<th>OS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ov</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.001 (0.872-1.149)</td>
<td>0.990 (0.887-1.095)</td>
<td>0.972 (0.863-1.095)</td>
<td></td>
</tr>
<tr>
<td>Ob</td>
<td>1.156 (0.999-1.339)</td>
<td>1.153 (1.006-1.276)</td>
<td>1.160 (1.048-1.290)</td>
</tr>
<tr>
<td>Chemo</td>
<td>1.074 (0.859-1.326)</td>
<td>1.073 (0.844-1.350)</td>
<td>1.074 (0.842-1.298)</td>
</tr>
<tr>
<td>Endo</td>
<td>1.001 (0.872-1.149)</td>
<td>0.990 (0.887-1.095)</td>
<td>0.972 (0.863-1.095)</td>
</tr>
<tr>
<td>Both</td>
<td>1.001 (0.872-1.149)</td>
<td>0.990 (0.887-1.095)</td>
<td>0.972 (0.863-1.095)</td>
</tr>
</tbody>
</table>

**P1-08-04**

**Obesity, Adjuvant Therapy, and Survival Outcomes in Early-Stage Breast Cancer.**

Jiralerspong S, Wang T, Rimawi MF, Nangia JR, Schiff R, Giordano SH, Pollak MN, Chenault CC, Osborne CK, Hilsenbeck SG. Baylor College of Medicine, Houston, TX; M.D. Anderson Cancer Center, Houston, TX; McGill University, Montreal, Canada

**BACKGROUND:** Obesity has risen to epidemic proportions and is associated with worse breast cancer (BC) prognosis in most studies. However, the effects of obesity according to adjuvant therapy choice are largely unknown. To address this issue, we examined the relationship between body mass index (BMI), adjuvant therapy, and survival outcomes in a large cohort of early-stage BC patients.

**METHODS:** We retrospectively studied patients from the Baylor Breast Center Tumor Bank treated from 1970-1995. Patients were divided into 3 BMI classes: normal/underweight (N, BMI<25), overweight (Ov, BMI 25-30), obese (Ob, BMI≥30); and 4 treatment groups: no adjuvant therapy, chemotherapy (mainly CMF), endocrine therapy (mainly tamoxifen), both chemo- and endocrine therapy. Time-to-recurrence (TTR), disease-free survival (DFS) and overall survival (OS) were estimated by the Kaplan-Meier method and compared among groups via the log-rank test. Multivariate analysis was conducted via Cox proportional hazards models.

**RESULTS:** There were 4,368 patients. Median age was 58. 74% were postmenopausal. 72% had stage I-II disease, 28% stage III. 76% were estrogen receptor (ER)-positive, 24% ER-negative. Patients distributed into BMI classes as follows: N 48%, Ov 30%, Ob 22%. Higher BMI was associated with postmenopausal status and increasing age, tumor size, positive lymph nodes, and stage, as well as a higher likelihood of receiving treatment. Median follow-up was 5 years. Kaplan-Meier analysis showed that TTR was significantly shorter in the Ov and Ob groups as compared to the N group (p=0.019), due to distant (p=0.001) rather than local (p=0.970) recurrences. DFS was also significantly worse in the Ov and Ob groups (p=0.002), as was OS (p=0.001). The Table shows the hazard ratios for the various survival outcomes after adjustment for age, tumor size, nodal status, and treatment groups. For all patients, TTR, DFS, and OS were significantly worse in the Ob vs. N groups. TTR and DFS were significantly worse in the chemo treated Ob vs. N groups. DFS and OS were significantly better in the endo treated Ov vs. N groups.

**DISCUSSION:** In this large cohort of BC patients, survival outcomes (TTR, DFS, OS) were significantly worse in the obese group. This remained true after adjustment for multiple factors. Obesity was associated with worse survival outcomes in the chemo treated (CMF) group. Overweight was associated with postmenopausal status and increasing age, tumor size, positive lymph nodes, and stage, as well as a higher likelihood of receiving treatment. Median follow-up was 5 years. Kaplan-Meier analysis showed that TTR was significantly shorter in the Ov and Ob groups as compared to the N group (p=0.019), due to distant (p=0.001) rather than local (p=0.970) recurrences. DFS was also significantly worse in the Ov and Ob groups (p=0.002), as was OS (p=0.001). The Table shows the hazard ratios for the various survival outcomes after adjustment for age, tumor size, nodal status, and treatment groups. For all patients, TTR, DFS, and OS were significantly worse in the Ob vs. N groups. TTR and DFS were significantly worse in the chemo treated Ob vs. N groups. DFS and OS were significantly better in the endo treated Ov vs. N groups.
P1-08-05
Age and Survival in Women with Early Stage Breast Cancer: An Analysis Controlling for Tumor Subtype.
Partridge AH, Hughes ME, Ottesen R, Wong Y-N, Edge SB, Theriault RL, Blayney DW, Niland JC, Winer EP, Weeks JC, Tamimi RM. Dana-Farber Cancer Institute, Boston, MA; City of Hope, Duarte, CA; Fox Chase Cancer Center, Philadelphia, PA; Roswell Park Cancer Institute, Buffalo, NY; University of Texas MD Anderson Cancer Center, Houston, TX; Stanford Cancer Center, Palo Alto, CA; Brigham and Women’s Hospital, Boston, MA

Background: Previous research has suggested that young age at diagnosis is an independent risk factor for breast cancer recurrence and death in women with early stage breast cancer. However, young women are more likely to have aggressive subtypes of breast cancer. No prior studies have adequately controlled for tumor phenotype, including HER-2/neu (HER2) status, in particular. Recent evidence has suggested that the prognostic effect of young age varies by tumor subtype.

Methods: We examined data from women with newly diagnosed Stage I-3 breast cancer presenting to one of 8 NCCN centers between January 2000 and December 2007. Multivariate Cox proportional hazards models were used to assess the relationship between age and breast cancer specific survival, controlling for known prognostic factors and treatment. In addition, we conducted stratified analyses by estrogen receptor (ER) and HER2 status.

Results: 19,633 women with Stage I-3 breast cancer eligible for analysis including 2,177 (11%) who were age 40 years or younger at diagnosis. Younger women were more likely to be non-white or Hispanic, more educated, employed, and to have higher stage, high grade, ER-negative, progesterone receptor (PR) negative, and HER2-positive disease, and treated with chemotherapy and trastuzumab (all variables P< 0.0001 by Chi-Square test). 5-year survival among younger women was 94.1% (95% Confidence Interval [CI] 92.9-95.3) and 96.3% (95% CI 95.9-96.6) for older women. In a multivariate Cox proportional hazards model controlling for sociodemographic, disease, and treatment characteristics, women age <40 or younger at diagnosis had increased mortality compared to older women (Hazard Ratio [HR] 1.26, 95% CI 1.02-1.56). In stratified analyses, age 40 or less was associated with increased mortality among women with ER-positive disease (HR 1.44, 95% CI 1.01-2.05), but was not among those with ER-negative disease (HR 1.15, 95% CI 0.85-1.55). Younger age was associated with a statistically significant increase in mortality among women with HER2-negative disease (HR 1.29, 95% CI 1.00-1.68), but this difference did not reach statistical significance among those with HER2-positive disease (HR 1.30, 95% CI 0.82-2.09).

Conclusions: The effect of age on short-term survival of women with early breast cancer appears to vary by breast cancer subtype, particularly ER status. Further research to elucidate differences in breast cancer biology and efficacy of therapy within tumor types by age is warranted.

P1-08-06
Breast Cancer among Patients with Diabetes, Obesity and Abnormal Blood Lipids – A Population-Based Register Study in Sweden.
Olsson H, Attner B, Landin Olsson M, Lithman T, Noreen D. Clinical Sciences, Lund University, Lund, Sweden

Objective: To study how the incidence of breast cancer is related to diabetes, obesity or abnormal blood lipids.

Methods: Diagnosis of diabetes, obesity or abnormal blood lipids was studied 0-10 years prior to the diagnosis of cancer in 2724 cases of cancer and in 20542 controls matched regarding age, sex and domicile in a population based material. Diagnoses were obtained by using out and inpatient population based registries. Also the use of glargine and metformin was studied in relation to cancer risk in diabetic patients using the national pharmacy prescription registry. Conditional logistic regression was used for the analyses.

Results: Diabetes was significantly more common prior to diagnosis in patients with breast cancer with diabetes diagnosed 0-4 years prior to the cancer diagnosis. The findings remained after adjusting for obesity and high blood lipids. Obesity was significantly more common in patients with breast cancer above the age of 60 years in those where obesity was diagnosed close to the diagnosis of cancer. High blood lipids were significantly less common in patients with breast cancer close to diagnosis.

Glarigine use was associated with a doubled risk 2.88 (1.15-6.64) and metformin use with a lower risk of cancer in diabetic patients 0.92 (0.82-1.09).

<table>
<thead>
<tr>
<th>Diabetes</th>
<th>Obesity</th>
<th>Blood lipids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast cancer A</td>
<td>1.18 (0.99-1.40)</td>
<td>0.70 (0.46-1.08)</td>
</tr>
<tr>
<td>Breast cancer B</td>
<td>1.37 (1.18-1.71)</td>
<td>0.79 (0.52-1.19)</td>
</tr>
<tr>
<td>Breast cancer C</td>
<td>1.11 (0.88-1.40)</td>
<td>0.79 (0.55-1.43)</td>
</tr>
</tbody>
</table>

Analysis Controlling for Tumor Subtype.

Breast Cancer Diagnosis and Chemotherapy Initiation: A SEER-Medicare Analysis.
Rugo H, Taylor D, Sanon M, Clements K, Balu S, Faria C, Teitelbaum A. University of California San Francisco; OptumInsight; Eisai Inc.

Objective: Use SEER-Medicare data to identify US women with metastatic breast cancer (MBC) treated (tx) with chemotherapy (CTX) and evaluate survival.

Methods: Key inclusion criteria were women diagnosed (dx) with breast cancer (BC) in 2001-2005 with 1) Medicare enrollment 12 mo prior to dx through follow-up (2008) or death; 2) an indication of tx with CTX at any time after initial BC diagnosis (any stage). Dx of MBC between 2001-2006. MBC dx date was defined as the initial BC dx date if the patient had “distant” disease at entry into SEER or the date of first appearance of secondary malignancy in claims data (ICD9 codes 197.0-198.1, 198.3-198.7, 198.82-198.89) during follow up, at least 2 mo after initial dx. CTX date was defined as the date of first receipt of a CTX agent commonly used to treat BC after the MBC date. Data on oral medications (e.g., most hormone therapies, capcitabine) were not available. Kaplan-Meier survival analyses were performed to describe survival after MBC dx (patients were censored at end of follow-up). Survival was assessed both from the date of MBC and receipt of first intravenous (IV) chemo. Subgroup analyses were performed based on age, type of MBC dx and estrogen receptor (ER) status.
Results: Patient demographics and survival are shown in the table.

<table>
<thead>
<tr>
<th>Median survival by patient characteristics</th>
<th>From BRC date</th>
<th>From first IV chemo date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall Survival (OS)</td>
<td>Months</td>
<td>N</td>
</tr>
<tr>
<td>&lt; 14</td>
<td>21.6</td>
<td>5805</td>
</tr>
<tr>
<td>15 - 39</td>
<td>55.1</td>
<td>1599</td>
</tr>
<tr>
<td>All first diagnosis</td>
<td>27.9</td>
<td>468</td>
</tr>
<tr>
<td>ER+</td>
<td>27.3</td>
<td>1760</td>
</tr>
<tr>
<td>ER-</td>
<td>22.7</td>
<td>932</td>
</tr>
<tr>
<td>Unknown</td>
<td>21.8</td>
<td>3133</td>
</tr>
</tbody>
</table>

Conclusions: Despite recent advances in treatment options, median survival following a dx of MBC is less than two years, and is approximately 16 months after start of IV CTX. Survival is shorter for patients who develop MBC after an initial breast cancer dx compared to those with MBC at diagnosis, and for those with ER-negative tumors. In order to improve clinical outcomes, it is critical to pursue a better understanding of tumor biology and appropriate use of new agents.

P1-08-08
Higher Prediagnostic Serum 25-Hydroxyvitamin D is Associated with Substantially Lower Incidence of Breast Cancer: Prospective Study.
Mohr SB, Gorham ED, Garland CF, Grant WB, Baggerly L. University of California San Diego, La Jolla, CA; Sunlight and Nutrition Research Center, San Francisco, CA; GrassrootsHealth, San Diego, CA

Background: Recent studies have found lower risk of breast cancer in women with higher serum concentrations of 25-hydroxyvitamin D (25(OH)D), with few exceptions. To help resolve remaining differences, a nested case-control study was conducted.

Material and Methods: Serum was provided by the Department of Defense Serum Repository for 600 new cases of breast cancer and 600 controls individually matched on age ±6 months; date blood was drawn ±2 days; and active-duty status; and were analyzed for 25(OH)D (25[OH]D), with few exceptions. To help resolve remaining differences, a nested case-control study was conducted.

Results: Race-adjusted odds ratios for breast cancer, from lowest to highest quintile, from lowest to highest, were 11, 18, 24, 30 and 42 ng/ml. The trend was similar after adjustment for race. The serum 25(OH)D concentration was high (42 ng/ml) had approximately 2-3 times lower risk of breast cancer in the lowest and highest 25(OH)D quintiles was significant.

Discussion: Consistent with previous research, women whose serum 25(OH)D concentration was high (42 ng/ml) had approximately half the risk of breast cancer as those whose serum 25(OH)D was low (11 ng/ml). The trend was similar after adjustment for race. The favorable association of serum 25(OH)D with risk of breast cancer apparently was strongest during the final few doublings of the tumor mass preceding diagnosis in this population. (An alternative, far less likely, interpretation, is that the tumor mass might adversely influence 25(OH)D concentration). A serum 25(OH)D concentration of 42 ng/ml can be achieved with vitamin D, intake of 4000 IU/day. This is the safe daily upper level intake of the National Academy of Sciences-Institute of Medicine (NAS-IOM December 2010 monograph). While further research would be beneficial, such intake should be recommended as a useful tool for prevention of breast cancer. Serum 25(OH)D should be monitored on a routine basis, when feasible, to ensure that at least 40 ng/ml is maintained throughout the year, and 150 ng/ml is not exceeded. This hygienic measure could prevent approximately 50% of breast cancer in the US. (The views expressed in this abstract are those of the authors and do not represent positions of the Department of the Navy, Department of the Army, the Department of Defense or the US Government.)

P1-08-09
Increased Mortality in Swedish Women Diagnosed with Breast Cancer during and Shortly after Pregnancy.
Johansson ALV, Andersson TM-L, Cnattingius S, Hsieh C-C, Lambe M. Karolinska Institutet, Stockholm, Sweden; University of Massachusetts Medical School, Worcester; Regional Oncologic Center, Uppsala, Sweden

Background: The incidence of pregnancy-associated breast cancer (PABC), i.e. breast cancer diagnosed during pregnancy or within two years after delivery, is increasing in Sweden. Results from epidemiological studies suggest that women with PABC have worse prognosis than women of same age and no recent birth. We studied the mortality in women with PABC, compared to women with a diagnosis of breast cancer not close to pregnancy, and in relation to when the PABC diagnosis occurred (during pregnancy, different postpartum periods). We also studied whether mortality varied over time since diagnosis.

Materials and Methods: We used data from the Swedish National Cancer Register to identify all women diagnosed with breast cancer between ages 15-44 years during 1963-2002 (n=15,729). The study cohort was linked to the Multi-Generation Register to obtain information on childbirths and when they occurred. We estimated mortality rates among women with and without PABC, and subdivided by time of detection among PABC patients (PABC during pregnancy, or during months 1-3, 4-6, 7-12, 13-18, 19-24 after delivery). Hazard ratios (HR) with 95% confidence intervals (CI) were estimated using the novel method of flexible parametric survival models. These models use splines to model the baseline hazard rate, and are able to pick up more subtle time-dependent effects than the more commonly used piecewise splitting of the timescale (for example in Poisson regression).

Results: Women with PABC had higher mortality than women diagnosed at the same age and calendar period and with no recent birth. In women with PABC (n=1,110), 46% died during follow-up, compared to 34% of the non-PABC patients (n=14,611). The mortality in both groups peaked at around two years after diagnosis, with the highest peak occurring in women diagnosed 4-6 months after delivery (HR=3.8, 95% CI 2.4-5.9). Also, among women diagnosed during pregnancy the mortality was higher compared to women diagnosed further away from delivery. An increased mortality among women with PABC remained up to 10 years after diagnosis.

Discussion: Women with PABC had a poorer prognosis compared to women with breast cancer and no recent birth. The worse prognosis may be due to diagnostic delays or tumour promoting hormonal boosts or changes in the tumour micro-environment post-partum. Taken together, our findings indicate that women with PABC represent...
a high-risk group of young breast cancer patients that may require special management. Also, by applying novel statistical methods we were able to detect time dependent effects in that the elevated mortality associated with PABC varied by timing of diagnosis and time since diagnosis.

**P1-08-10**

**Invasive Lobular Breast Cancer – No Increased Risk of Contralateral Disease.**

Langlands F, Morgen K, Kearins O, Burns R, Dowdell D. The General Infirmary at Leeds, Leeds, United Kingdom; The University of Birmingham, Birmingham, United Kingdom; Level 6, Besley Wing (Institute of Oncology), Leeds, United Kingdom; St James University Hospital, Leeds, United Kingdom

**Background:** Invasive lobular carcinoma (ILC) is the second most common type of breast cancer accounting for up to 14% of invasive breast cancers. Worldwide the incidence of breast cancer is increasing each year. Large population based studies using the Surveillance, Epidemiology, and End results (SEER) data have shown that the incidence of ILC has increased from 1977 to 1995. ILC has historically been thought to be associated with an increased risk of developing contralateral breast cancer and this belief may, in part, be responsible for the increasing trend towards contralateral prophylactic mastectomy.

**Methods:** All female patients diagnosed with breast cancer during the time period 1998-2003 were identified from two large cancer registries, Northern and Yorkshire Cancer Registry and Information Service (NYCIRS) and West Midlands Cancer Intelligence Unit (WMCIU). All patients diagnosed with either Infiltrating duct(adeno) carcinoma, microinvasive ductal carcinoma, infiltrating ductular carcinoma (all classified as ‘ductal’) or lobular (adenocarcinoma were included. Patients with a mixed type of breast cancer were excluded from the analysis. Follow-up was complete until October 2010. Data were compiled for diagnosed contralateral breast cancer of any histological type.

**Results:** Of the 32,735 patients with invasive ductal cancer 898 (2.74%) developed a contralateral breast cancer. In comparison 166 (3.1%) of 5397 patients with lobular breast cancer developed a contralateral breast cancer. The median time to first contralateral event was equivalent for both morphologies (ductal 40 months and lobular 39 months).

**Conclusion:** This study suggests that there is no increased risk for developing a contralateral breast cancer in patients diagnosed with an invasive lobular breast cancer.

**P1-08-11**

**Differences in Recurrence Dynamics between Lobular and Ductal Invasive Breast Cancer.**

Siesling S, Kwast ABG, Grandjean I, Ho V, van der Sangen MJC, Menke-Pluymer MBE, Tjan-Heijnen VCG. Comprehensive Cancer Centre the Netherlands, Utrecht, Netherlands; Catharina Hospital, Eindhoven, Netherlands; Erasmus Medical Centre, Rotterdam, Netherlands; Maastricht University Medical Centre, Maastricht, Netherlands

**Introduction:** Many studies investigated disease free survival after breast cancer treatment. A few studies focused on patterns of recurrence risk over time. However, they ignored the impact of histology. This study aimed to determine differences in recurrence dynamics on population-based data between patients with a ductal breast cancer (DBC) and lobular breast cancer (LBC).

**Methods:** All surgically treated women diagnosed in 2003-2004 with invasive DBC or LBC, with no distant metastases or second primary breast cancer were selected from the Netherlands Cancer Registry. The recurrence patterns were studied using the life-table method to estimate the hazard rate for the first recurrence with the worst prognosis (locoregional (LRR) or distant metastases), that is, the conditional probability of manifesting recurrence in a time interval, given that the patient is clinically free of any recurrence at the beginning of the interval. The wilcoxon test was used to determine differences in recurrence patterns between DBC and LBC.

**Results:** Of 16,231 women identified, 87% had DBC and 13% LBC. LRR was found in 4% of the DBC and in 3% of the LBC patients, whereas metastases were found in 11% and 10% of patients, respectively. Notably, in DBC patients a peak in recurrence rate was seen between 1-2 years after diagnosis, both for LRR and distant metastases. On the other hand, in LBC patients the recurrence pattern did not show major peaks: after 2 years the curve revealed an almost steady level for LRR and distant metastases. This recurrence pattern was significantly different (P<0.001). Moreover, the influence of age, stage and treatment on recurrence differed between DBC and LBC. We also noticed a difference in localization of metastasis.

**Conclusion:** Our study showed an overall equal number of recurrences after DBC and LBC, but with a totally different recurrence pattern, which could have implications on follow-up of the patients.

**P1-08-12**

**Hormonal Therapy Compliance and Mortality in Metastatic Breast Cancer.**

Barron TI, Kennedy MJ, Sharp L, Bennett K. Trinity College, University of Dublin, Dublin, Ireland; St James’s Hospital and Trinity College Dublin, Dublin, Ireland; National Cancer Registry Ireland, Cork, Ireland

**Methods:** A retrospective analysis was conducted using linked pharmacy claims and cancer registry data from the population-based National Cancer Registry Ireland (NCRI). Women with stage IV breast cancer at initial diagnosis (2004-2006), who received HTx as 1st line treatment for ≥90 days were identified (tamoxifen, toremifene, fulvestrant, anastrozole, letrozole, exemestane). Compliance was calculated over the time that patients remained on HTx i.e. from the date of treatment initiation to the earliest of either HTx discontinuation or end of follow up (31/12/07, 3 years or death). Patients discontinuing HTx were assigned their compliance rate at this time for the remainder of follow-up. In the primary analysis Cox proportional hazard models with late entry at 90 days post HTx initiation and time varying compliance (defined dichotomously as: low < 80%, high ≥ 80%), were used to estimate hazard ratios (HR) and 95% confidence intervals (CI) for all-cause and breast cancer specific mortality. Secondary analyses of (a) time to treatment discontinuation and (b) compliance in the first 90 days of treatment were conducted. All models were adjusted for age, tumor grade, number/site of metastases, estrogen, progesterone and human epidermal growth factor 2 receptor status.

**Results:** 359 women with stage IV breast cancer at diagnosis were identified. Of these, 151 received HTx as 1st line treatment. Median HTx compliance was 84.8%, (range 19.4%-100%; interquartile range 75.5%-100%). Demographic, tumor and treatment characteristics were similar between low (n=51) and high (n=100) compliance groups. The number and distribution of metastatic sites at diagnosis was also similar (low/high compliance: bone only 70.6%/70.0%, lung only 17.6%/18.0%, liver only 11.8%/14.0%, brain only 0.0%/0.0%, other 13.7%/17.0%, multiple sites 15.7%/18.0%). Compliance rates for both compliance groups increased marginally over time.
Unadjusted all-cause mortality rates were 143 and 359 deaths/1000 woman years for low and high compliance respectively. Low compliance was associated with a 90% increased risk of all-cause (HR 1.90; 95%CI 1.06, 3.41) and breast cancer specific (HR 1.91; 95%CI 1.03, 3.55) mortality. In the secondary analyses low compliance was associated with a shorter time to HTx discontinuation (HR 1.97; 95%CI 1.01, 3.84). Low compliance within the first 90 days of treatment was associated with non-significantly increased mortality (all-cause HR 1.57; 95%CI 0.88, 2.80; breast cancer specific HR 1.58; 95%CI 0.85, 2.93).

Discussion: This study demonstrates, for the first time to our knowledge, a significant association between low HTx compliance and increased mortality in patients with MBC. In addition, the observed association between compliance and treatment duration is consistent with a reduced time to disease progression in low compliance patients. The findings do not appear to be related to either the burden of metastatic disease at diagnosis or to increasing disease severity causing lower compliance over time. These results suggest that the development and evaluation of effective interventions to support HTx compliance in patients with MBC is warranted.

**P1-08-13**

**Determinants of Risk, Characteristics and Prognosis of Breast Cancer Occurring after Hodgkin Lymphoma.**


**Background**

Several studies have evaluated the excess of breast cancer (BC) risk after Hodgkin lymphoma (HL) but none has reported of all the aspects of BC occurring after HL including determinants of risk, comparison with other BC profile, treatment and outcome. This is the aim of our study.

**Patients and Methods**

We used data from the Surveillance, Epidemiology and End Results (SEER) Programme of the National Cancer Institute. To assess the risk and determinants of BC after HL we included all 9’620 women with first primary HL diagnosed between 1973 and 2007 and calculated age and period standardized incidence ratio (SIR) of BC as compared with general population. We also compared tumour characteristics and treatment between BC after HL (n=316) vs. all other first primary BC occurring during the same period (n=450’413) by logistic regression. Finally, we compared breast cancer specific mortality between the two groups by Cox model.

**Results**

Overall, HL patients had an increased risk of developing BC (SIR: 2.4, 95% confidence interval [CI]: 2.2-2.7); the risk decreased with increasing age at HL, and disappeared when HIV occurred after 50 years. BC risk was the same among those treated with or without radiotherapy until 15 years after HL and then an excess appeared in the irradiated group and persisted over 30 years after diagnosis of HL. Compared with other BC, BC after HL occurred at younger age (Adjusted odds ratio [OR] for age 40-59 vs. < 40 years 0.25, 95%CI: 0.20-0.33), was diagnosed at an early stage (OR for regional extension vs. localized 0.63, 95% CI: 0.42-0.89), expressed less frequently hormone receptors (OR for negative vs. positive status: 1.34, 95%CI: 0.99-1.81), was located more frequently in external quadrants (OR for internal vs. external: 0.61, 95%CI: 0.42-0.89), and received less frequently radiotherapy (OR for use vs. non use: 0.30, 95%CI: 0.22-0.42). The last two results were limited to patients who had received radiotherapy for HL. BC after HL presented higher breast cancer mortality (Hazard ratio: 1.36, 95%CI: 1.05-1.76) after adjustment for patients, tumour and treatment characteristics.

**Conclusion**

This study provides valuable results on various aspects of BC occurring after HL. In particular, we found earlier stage at diagnosis of BC probably linked to higher surveillance, more aggressive tumours probably due to iatrogenic effect of HL treatment, more frequent occurrence in outer quadrants less protected during mantel radiation. Also, this study confirms the poorer prognostic of those BC not explained by differences in patient, tumour or treatment characteristics.

**P1-08-14**

**Arsenic Methylation Is Associated with Breast Cancer Risk in Northern Mexico.**

López-Carrillo L, Hernández-Ramirez U, Torres-Sánchez L, Gandolfi JA, Ornelas-Aguirre JM, Cebrián ME. Mexico National Institute of Public Health, Cuernavaca, Morelos, Mexico; University of Arizona, Tucson, AZ; Hospital de Especialidades. Instituto Mexicano del Seguro Socia, Cd. Obregón, Sonora, Mexico; Centro de Investigación y Estudios Avanzados (Cinvestav), Mexico, DF, Mexico

**Background:** Arsenic (iAs) exposure has been associated with several types of cancer. However, most studies to date have not yet implicated iAs as a cofactor for breast cancer (BC). BC incidence and mortality are 3-fold higher in Northern Mexico (NM) than in the rest of the country. iAs levels in drinking water are a source of concern since they have been above international standards. We hypothesized that iAs exposure and individual Arsenic-methylation ability are potential cofactors for BC risk.

**Material and Methods:** We investigated 840 BC incident cases and 973 controls from a population-based case-control study performed in NM. Women were directly interviewed about dietary and reproductive BC covariables. iAs metabolites in urine were determined by HPLC/ICP-MS. Methylation capacity was assessed by calculating the percentage of iAs species and primary [MMA(V)/(As(III)+As(V)) (PMI) and secondary [DMA(V)/MMA(V)] methylation indexes (SMI).

**Results:** The range of total urinary Arsenic was 0.4-303.9 ug/L. Most women (91%) had values above 30 ugAs/L. %MMA(V) was significantly higher in BC cases while %DMA(V) was significantly lower. PMI was positively associated with BC (OR T3 vs T1=1.47; CI95%=1.17-1.84; p for trend=0.001), while SMI was negatively associated (OR T3 vs T1=0.54; CI95%=0.42-0.68; p for trend=0.001).

**Discussion:** As exposure may pose a risk for BC, particularly in women with higher capacity to methylate iAs to MMA(V) and lower capacity to further methylate MMA(V) to DMA(V). This is consistent with studies indicating that the proportion of MMA(V) in urine was positively associated whereas that of DMA(V) was inversely associated with the risk of skin, bladder and lung cancer. Further research is needed about the temporal relationships between disease and methylation capacity and the presence of potential association modifiers, including environmental and genetic factors involved in one carbon metabolism.
PI-08-15
Pattern of Cardiac Monitoring and a Risk of Trastuzumab Associated-Cardiac Dysfunction in a Clinical Practice: A Population Based Study.
Lee-Ying R, Ubhi C, Roberts S, Lim H, Bhatt H, Gesy K, Ahmed S. University of Saskatchewan, Saskatoon, SK, Canada; University of Saskatchewan; Saskatchewan Cancer Agency

Background: Adjuvant trastuzumab therapy (ATT) in women with early-stage HER2+ breast cancer has been associated with significant reduction in the disease recurrence and mortality. Cardiac dysfunction (CD) is a known serious adverse effect of ATT. Although periodic cardiac monitoring is recommended during ATT, little is known about pattern of cardiac monitoring and incidence of CD in a clinical setting. The study aimed to determine extent of cardiac monitoring and rate of CD during ATT and to identify factors correlated with CD.

Methods: Medical records of women with localized HER2+ breast cancer diagnosed between the years 2005-2007 in the province of Saskatchewan were reviewed. Women with advanced or recurrent disease or if they were treated in the setting of an adjuvant trastuzumab trial were excluded. A logistic regression analysis was performed to determine various clinical variables correlated with CD.

Results: A total 116 eligible women with median age of 54 yrs (range: 27-74) and median BMI of 27 (range: 14-47) were identified. 40% had a cardiac risk factor & 30% were premenopausal. 51% had node positive & 53% had HER2+ breast cancer. 92% received anthracycline-based chemotherapy and 23% received sequential ATT. Of 62 patients with ER/PR+ breast cancer, 61% received adjuvant aromatase inhibitors. Baseline cardiac assessment was performed in 93% women, 98% women underwent periodic cardiac monitoring during ATT, 55% had monitoring performed at the interval of 3-4 months & 82% women had monitoring performed at the interval of 3-6 months. Mean baseline cardiac ejection fraction (EF%) prior to the commencement of chemotherapy and ATT were 65% & 63.9% respectively (p<0.05). CD was observed in 32 (28%) women and only 4% were asymptomatic. Trastuzumab was interrupted in 34%, and was discontinued in 20% women. Of 32 women with CD, 59% were referred to a cardiologist and 53% were treated with medication. CD was reversible in 84% cases. On multivariate analysis adjuvant aromatase inhibitor therapy was significantly correlated with cardiac dysfunction (Odds ratio 6.9 [95% CI: 1.4-33.0]). During the follow up period 18% women developed recurrent disease and 16% were died.

Conclusions: Our results confirm high compliance with cardiac monitoring, though not as frequently as recommended in the clinical trial setting. Overall the rate of symptomatic decline in cardiac function was similar to the rate reported in the clinical trials, however, a relatively higher incidence of asymptomatic decline in the left ventricle EF% was noted. Among various variables examined, adjuvant aromatase inhibitor therapy was associated with an increased risk of CD.

PI-08-16
Benign Breast Disease (BBD) and Breast Cancer in African American Women.
Fehmi RA, Cote M, Ruterbusch J, Alosh B, Bandypadhyay S, Albashiti B, Frost MH, Hartmann LC, Vieszcher DW. Wayne State University/Karmanos Cancer Institute/DMC, Detroit, MI; Wayne State University/Karmanos Cancer Institute, Detroit, MI; Mayo Clinic Cancer Center, Rochester, MN

African American (AA) women have higher mortality rates from breast cancer (BrCa) and are diagnosed at younger ages than their Caucasian counterparts. Women who have had a benign breast biopsy are at increased risk of the disease, although less is known about the risk of BrCa associated with benign breast disease in AA women. We examined 1428 breast biopsies from AA women which occurred from 1997-2000 and assessed various pathologic markers including: apocrine metaplasia, ductal hyperplasia including atypia, evidence of cysts, duct ectasia, fibrosis, intra-ductal papilloma, sclerosing adenosis, columnar alteration, and involution (atrophy). These women were followed for later BrCa through the Metropolitan Detroit Cancer Surveillance System, part of the Surveillance, Epidemiology and End Results (SEER) program through 2008. Women who developed BrCa were compared to those in the cohort who did not, and to other AA women with BrCa in the SEER registry. AA women in our study were also compared to Caucasian women in the Mayo Clinic cohort. Differences in variables were assessed by chi-squared tests and 95% confidence intervals. Of the 1428 biopsies, 52 (3.6%) subsequent incident breast cancers were reported in SEER. The mean age at diagnosis was 59, and the mean time from biopsy to BrCa diagnosis was 6.1 years. Individuals with atypical ductal hyperplasia at biopsy were more likely to develop breast cancer (n=7, 13.5%, p<0.01). No other pathologic variables were associated with increased risk. Women in our cohort with breast cancer did not differ from AA in the SEER database with respect to age at diagnosis, stage at diagnosis, or receptor positivity. Compared to the Caucasian women in the Mayo Clinic BBD cohort, AA women in our study were younger at biopsy (p<0.01), but had similar percentages of involution and atypia (p=0.50 and 0.15, respectively). Our preliminary findings among a relatively small group of AA women with prior benign breast biopsies and incident breast cancers suggest that results from the Mayo Cohort study are likely to apply to AA populations.

PI-08-17
Pregnancy-Associated Breast Cancer Does Not Have a Worse Outcome in the 4912 Women with Breast Cancer of the AMAZONA Project.
Liedke PER, Szymonifka J, Simon SD, Barrios CH, Bines J, Finkelstein D, Goss PE. Massachusetts General Hospital, Boston, MA; Universidade Federal de São Paulo (UNIFESP), São Paulo, Brazil; Pontificia Universidade Católica do RS (PUCRS), Porto Alegre, Rio Grande do Sul, Brazil; Instituto Nacional do Cancer (INCA), Rio de Janeiro, Brazil; Brazilian Breast Cancer Study Group (GBECAM), São Paulo, Brazil

Introduction: Pregnancy traditionally is considered a protective factor for breast cancer. Recent data suggests that pregnancy-associated breast cancer (PABC), a distinct biologic variant possibly related to breast involution, can occur up to 10 years post-partum and may carry a worse prognosis than that of age matched sporadic or nulliparous breast cancer. The Amazona project is a retrospective cohort of 4,912 Brazilian women with breast cancer that has previously reported on worse outcomes of patients according to type of institution where treatment was received (San Antonio 2009 abstr. 306). We have assessed the outcomes of PABC in the Amazona cohort.

Objectives: 1- To identify whether women who were diagnosed with breast cancer up to 10 years after their first pregnancy had worse disease free survival (DFS) and overall survival (OS) than nulliparous women (NW); 2- to assess if age at first pregnancy is related to age of breast cancer diagnosis and worse DFS or OS; 3- to assess whether number of pregnancies is associated with worse DFS or OS; 4- to assess whether time from first pregnancy to diagnosis or age of first pregnancy are associated with histological grade, clinical stage or tumor expression of ER, PR, and HER2.
Methods: We analyzed 4836 women for whom parous history was available, in respect to DFS, OS, tumor clinical stage, histological grade, expression of ER, PR and HER2, according to age of first pregnancy, diagnosis up to 5 and 10 years after first pregnancy, and number of pregnancies, using up to 10 years as controls. Analysis of DFS and OS was done by Cox regression modeling adjusted for institution type, stage, ER, PR, HER2 and grade.

Results: Our cohort had 1996 nulliparous women and 2840 parous women. The median follow up was 28 months and there were 318 deaths and 735 recurrences. We did not find any correlation between PABC with DFS (5 year interval HR 1.15, 95%CI 0.43-3.07; 10 year interval HR 1.01, 95%CI 0.57-1.81) or OS (5 year interval HR 1.88, 95%CI 0.6-5.94; 10 year interval HR 0.5, 95%CI 0.73-3.09), nor was there a correlation between age at first pregnancy with age of breast cancer diagnosis. We also did not see any difference between age of first pregnancy and DFS or OS.

Women with 3 or more pregnancies had worse OS (HR 0.71, 95%CI 0.54-0.93) but not worse DFS (HR 0.93, 95%CI 0.76-1.13).Tumors diagnosed within 5 or 10 years from first pregnancy did not differ by grade, ER, PR, HER2, and clinical stage from those of NW. Women who had their first pregnancy after age 20 tended to have more ER+ tumor (OR 1.99, 95%CI 1.40-2.65), PR positive tumor (OR 1.40, 95%CI 1.06-1.87), and HER2 positive tumor (OR 1.85, 95%CI 1.22-2.79) tumors than NW.

Conclusions: In this large cohort of breast cancer patients from the diverse geographic and socioeconomic spectrum of Brazil we did not find any association between PABC or age of first pregnancy to DFS or OS. The association with worse OS but not DFS of women with 3 or more pregnancies might be due to confounding factors. PABC was not associated with worse clinical prognostic factors. Women who had their first pregnancy after age 20 were more likely to have ER+, PR+ and HER2+ tumors than nulliparous patients.

P1-08-18
Aspirin Exposure and Nodal Status at Diagnosis in Women with Stage I-III Breast Cancer.
Barron T, Sharp L, Bennett K, Visvanathan K. Trinity College, University of Dublin, Dublin, Ireland; National Cancer Registry Ireland, Cork, Ireland; Johns Hopkins School of Public Health, Baltimore, MD

Background: The recent Nurses’ Health Study reported an association between aspirin use and a reduction in breast cancer (BC) recurrence and BC-specific mortality. It has been suggested based on preclinical data that these effects may be due to the inhibition of tumor invasion and metastasis by aspirin. To test this hypothesis we examined the association between aspirin exposure prior to BC diagnosis and nodal involvement at diagnosis.

Methods: A retrospective analysis was conducted using linked pharmacy claims and cancer registry data from the National Cancer Registry Ireland. Women with an incident diagnosis of stage I-III BC between 2001-2006 and no prior invasive cancer were included. Women with a prescription for aspirin at any time in the year prior to diagnosis were defined as exposed. Multivariate logistic regression was used to estimate odds ratios (OR) and 95% confidence intervals (CI) for (a) N0 disease; (b) N1 disease (c) N1/2/3 disease at diagnosis in aspirin exposed (overall exposure & exposure quartiles by duration of use) versus unexposed women. All analyses were adjusted for age, tumor size/grade, ER, PR and HER2 receptor status. Analyses were repeated including women with stage IV BC at diagnosis. Survival analyses are ongoing.

Results: 4212 women with stage I-III BC at diagnosis were identified. 1086 (25.8%) had a supply of aspirin available to them in the year prior to diagnosis (median dose 75mg, interquartile range 75mg, 75mg). Women taking aspirin were older than those who did not (74.3 yrs/64.4 yrs p<0.05). There was no difference in tumor sizes, grade, ER, PR or HER2 receptor status between exposed and unexposed women. In the multivariate logistic regression analysis aspirin exposure was associated with significantly increased odds of an N0 tumor (Table 1; OR 1.27 95%CI 1.09, 1.48) and significantly decreased odds of an N1 or N1/2/3 tumor. Additionally, women taking aspirin for >95% of the year prior to diagnosis had the highest odds of an N0 tumor (Table1; OR 1.50 95%CI 1.14, 1.97 ). There was no difference in the odds of an N0 tumor for women taking aspirin for <40% of the prior year (OR 1.01 95%CI 0.78, 1.31). Inclusion of stage IV BC (n=789) in the analysis did not significantly alter these results.

P1-08-19
Withdrawn by Author

P1-08-20
Parity Interferes with the Effect of Age at Diagnosis on the Frequency Breast Cancers Are Triple-Negative.

Background: Epidemiologic studies show an age related decrease in the frequency of TN breast cancer, < 51 years at diagnosis. We compared the frequency of TN breast cancers between parous (N = 1271) and nulliparous (N = 312) women in three age categories (21 to 30 yrs, 31 to 40 yrs and 41 to 50 yrs). For statistical analysis we used a logistic regression model.

Results: Our cohort had 1996 nulliparous women and 2840 parous women. The median follow up was 28 months and there were 318 deaths and 735 recurrences. We did not find any association between PABC or age of first pregnancy to DFS or OS. The association with worse OS but not DFS of women with 3 or more pregnancies might be due to confounding factors. PABC was not associated with worse clinical prognostic factors. Women who had their first pregnancy after age 20 were more likely to have ER+, PR+ and HER2+ tumors than nulliparous patients.
### P1-08-21

**Demographic and Clinical Characteristics of Metastatic Breast Cancer Patients and Biomarker-Based Prevalence in the UK, Germany, France, Spain and Italy (EU-5).**

**Ganguli A, DeKoven M, Bonthapally V, Lee WC, Ray S. Abbott Laboratories, Abbott Park, IL; IMS Consulting Group, Alexandria, VA**

**Background:** Much peer-reviewed literature focuses on metastatic breast cancer (mBC) treatment regimens. However, research around mBC patients' demographic/clinical characteristics across Europe is limited. This study compared such mBC characteristics as well as biomarker-based prevalence across the EU-5.

**Methods:** IMS LifeLink™ Oncology Analyzer (OA) database, based upon practicing oncologist surveys, was used to identify mBC patients aged ≥18 between 01/2005-06/2010. The study investigated the distribution of mBC population based on age, biomarker, comorbidities and stage at diagnosis. This study also estimated the proportion of patients, and sites of metastasis, by lines of drug therapies (LOT).

**Results:** A total of 186,640 mBC patients were identified - Germany (30.2%), France (22.4%), UK (21.2%), Italy (17.7%) and Spain (8.4%). The majority of patients were aged 61-70yrs (24%-32%), except in Spain (22.3% aged 71-80yrs). Proportion of mBC patients with ≥1 co-morbid condition were highest in Germany (36.3%), followed by Spain (32.8%), UK (31.5%), Italy (27.6%) and France (20.1%), with diabetes (12.9-23.9%) and cardiac dysfunction (5.2-21.7%) being most prevalent. The distribution of mBC patients by biomarker status was 53.9% HER-/HR+, 17.8% HER+/HR+, 11.1% HER+/HR- and 17.2% triple negative, and was similar amongst the EU5 countries. The top three metastatic sites were bone (54.4%), lung (36.0%) and liver (32.7%), with proportion of bone metastasis increasing from 1st LOT (38.1%) to 4th LOT (69.6%). Almost 50% of the mBC patients were diagnosed at Stage IV, 14% at Stage III, 26% at Stage II and 6% at Stage I. Of all mBC patients, 93% received 1st LOT, 31% received 2nd LOT, 27% received 3rd LOT and 6.8% received 4th LOT.

**Conclusion:** In the EU-5, mBC patients were primarily elderly and HER-/HR+. The burden of bone metastasis was higher in later LOTs. Following the 1st LOT, fewer patients moved to subsequent LOTs. Following the 1st LOT, fewer patients moved to subsequent LOTs. The international BCAC has recently accepted to validate our UZ Leuven findings.

### P1-08-22

**Treatment Patterns and Clinical Outcomes in Elderly Patients with HER2-Positive Metastatic Breast Cancer from the registHER Observational Study.**

**Kaufman PA, Brafsky AM, Mayer M, Rugo HS, Tripathy D, Ulicickas Yood M, Feng S, Wang LI, Brammer MG, Yardley DA. Norris Cotton Cancer Center, Dartmouth-Hitchcock Medical Center, Lebanon, NH; University of Pittsburgh Cancer Center, Pittsburgh, PA; Patient Advocate, New York, NY; University of California San Francisco Comprehensive Cancer Center, San Francisco, CA; University of Southern California/Norris Comprehensive Cancer Center, Los Angeles, CA; EpiSource, LLC; Boston, MA; Boston University School of Medicine, Boston, MA; Genentech, Inc., South San Francisco, CA; Sarah Cannon Research Institute, Nashville, TN; Tennessee Oncology, PLLC, Nashville, TN**

**Background:** Data are lacking regarding treatment patterns and outcomes in elderly patients (pts) with HER2-positive (HER2+) metastatic breast cancer (MBC).

**Methods:** registHER is a large, observational cohort of pts with HER2+ MBC diagnosed within 6 months of enrollment. Pts (N=1,001) were followed until death, disenrollment, or June 2009 (median follow-up 27 months). In these analyses, pts were stratified into three groups based on age at MBC diagnosis: younger (<65 years), older (65-74 years), elderly (≥75 years). For Progression Free Survival (PFS) and Overall Survival (OS) analyses of 1st-line trastuzumab (T) vs. no T, older and elderly pts were combined due to small number of events in elderly. Hierarchical multivariate analyses were adjusted for baseline characteristics and treatments.

**Results:** ER/PR status was similar across age groups (Table 1). Elderly pts with HER2+ MBC had higher rates of underlying cardiovascular disease (CVD) than younger or older pts. In pts receiving T-based 1st-line treatment, elderly pts were less likely to receive chemotherapy (C), and more likely to receive T alone or combined with hormone therapy (HT). Central nervous system (CNS) events decreased with increasing age. In T-treated pts, incidence of left ventricular dysfunction (grade ≥3) was higher in elderly pts (20.1%) than in younger (21.4% [2.8%]) or older pts (21.4% [1.5%]). Across age groups, unadjusted median PFS (months) was significantly higher for pts treated with T in 1st-line than those who were not (<65 years T: 11.0; <65 years no T: 3.4; ≥65 years T: 11.7; ≥65 years no T: 4.8). In pts <65 years, unadjusted median OS (months) was significantly higher in T-treated pts: in pts ≥65 years, median OS was similar (<65 years T: 40.4, <65 years no T: 25.9; ≥65 years T: 31.2, ≥65 years no T: 28.5). In multivariate analyses, T in 1st-line was associated with significant improvement in PFS across age (Table 2). In OS, significant improvement was observed for pts <65 years; results were suggestive for pts ≥65 years.

**Conclusions:** Elderly pts (≥75 years) with HER2+ MBC in registHER had higher rates of underlying CVD than younger counterparts and received less aggressive treatment, including less 1st-line T. These population-based, real-world data suggest improved PFS with T as 1st-line therapy across all age groups.

### Table 1. Clinical characteristics of younger (<65 years), older (65-74 years) and elderly (≥75 years) pts

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<th>Parameter (%)</th>
<th>&lt;65 years (n=668)</th>
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<th>≥75 years (n=50)</th>
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<td>ER+ &amp; PR+</td>
<td>53.8</td>
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<td>Underlying CVD at baseline</td>
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<td></td>
<td></td>
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<tr>
<td>HER2+ 1st-line regimens</td>
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<td>29.2 (46.2)</td>
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<td>Any CNS event</td>
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**P1-08-23**

**Characteristics of De Novo Stage IV Breast Cancer Presentation and Comparison with Stage IV Disease Relapse after Adjuvant Therapy.**


**Background:** We hypothesized that patients diagnosed with stage IV breast cancer at presentation may have different disease characteristics than patients who develop relapsed stage IV disease after adjuvant therapy. To examine potential differences, we analyzed a prospectively collected data set for patients diagnosed at the Markey Cancer Center starting from the beginning of 2007 to date.

**Methods:** Out of 1089 patients, we identified 76 patients (7%) who presented with de novo stage IV disease and 40 patients (4%) who experienced systemic disease relapse after initial adjuvant therapy. We compared key variables between the two groups including patient age, tumor size, grade, estrogen receptor (ER) status, progesterone receptor (PgR) status, HER2, and sites of metastatic disease involvement. Statistical significance was determined using the Chi-square test, Fisher’s exact test, two-sample t-test, or Wilcoxon’s rank sum test as appropriate.

**Results:** Patients with de novo stage IV breast cancer were more likely to be older (median age 58.5 years, Range 28-88) than women with relapsed stage IV breast cancer (median age 53 years, Range 28-77), and this difference was statistically significant (p = 0.039). As compared to the group with relapsed disease, breast cancer presenting with de novo stage IV disease was more likely to be Grade 1/2 (43% vs. 18.4%, p=0.032), ER positive (69.7% vs. 47.5%, p = 0.019), and PgR positive (56.6% vs. 32.5%, p = 0.0136). Interestingly, de novo stage IV breast cancer was more likely to be HER2 positive than in patients with relapsed disease (27.5% vs. 10.26%, p=0.035). When available patient data prior to 2007 was included in the analysis, we observed no significant difference in the frequency of HER2 expression between the de novo and relapsed groups (27.4% vs. 19.4%, p = 0.26). In comparing sites of metastatic disease involvement, the de novo stage IV patient group was more likely to have bone metastasis than the relapsed disease patient group (68.4% vs. 35%, p = 0.0006). There were no significant differences in the frequency of other metastatic sites involved, including brain, liver, and lung.

**Conclusions:** In this breast cancer patient cohort, there was a relatively high percentage of women presenting with stage IV disease, likely reflecting the underserved nature of this patient population. Women with de novo stage IV breast cancer were more likely to be older, have ER/PgR positive disease, and have bone metastasis as the primary site of involvement. These characteristics may reflect delay in initial presentation rather than a biologically more aggressive disease phenotype. The higher relative frequency of HER2 positive cancer in the de novo stage IV patient group is likely related to the impact of adjuvant trastuzumab use on reducing the risk of relapse in HER2 positive breast cancer.

**Table 2. Multivariate* results for PFS and OS in younger (<65 years) and combined older and elderly (<65 years) pts (T in 1st-line vs. no T in 1st-line)**

<table>
<thead>
<tr>
<th></th>
<th>Hazard Ratio</th>
<th>95% CI</th>
<th>P-value</th>
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<td>&lt;65 years</td>
<td>0.46</td>
<td>0.32-0.69</td>
<td>&lt;0.01</td>
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<td>≥65 years</td>
<td>0.52</td>
<td>0.35-0.76</td>
<td>&lt;0.01</td>
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<tr>
<td>OS</td>
<td>0.59</td>
<td>0.46-0.77</td>
<td>&lt;0.01</td>
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<td>≥65 years</td>
<td>0.72</td>
<td>0.52-0.98</td>
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*Adjusted for race, ECOG performance status, serum albumin, ER/PR status, #metastatic sites, stage, underlying CVD, non-cardiac comorbidities, 1st-line C, HT

**P1-08-24**

**Clinical and Pathologic Characteristics of Diabetic Breast Cancer Patients in a Tertiary Care Safety Net Hospital.**

Loch MM, Eapen A, Ross AA, Rosenberg C, Blanchard RA. Boston University Medical Center, Boston, MA; Boston University, Boston, MA

**Background:** Recent studies have indicated an increased risk of breast cancer in women with diabetes, and an increase in disease specific mortality in patients with diabetes and breast cancer. Clinical and pathologic features of diabetic women with breast cancer have not been described. We examine tumor characteristics in diabetic women in our ethnically diverse tertiary care hospital that may help explain the increased mortality seen in diabetic women diagnosed with breast cancer.

**Methods:** We expanded a pre-existing database to include all women diagnosed with breast cancer from 1998-2011. From the electronic medical record we documented age, body mass index (BMI), place of birth, self-identified race/ethnicity, date of diagnosis, T, N, M stage, grade, ER, PR, HER2 expression, presence of diabetes (DM) or thyroid disease at diagnosis, recurrence and treatment data including neoadjuvant, surgical, adjuvant and radiation therapy and adequacy of treatment. We determined associations using contingency tables and multivariable logistic regression.

**Results:** 480 cases were identified. Patients were racially diverse: 35% white [W], 41% black [B], 14% Hispanic and 10% other. 23% had DM, 47% were obese (body mass index [BMI] >30). Age in DM vs. non-DM were 63.327 vs. 57.729 (p = < 0.0001) and BMI in DM vs. non-DM were 32.749 vs. 29.433 (p = 0.001). When exploring odds ratios (OR) of different tumor subtypes, none were statistically significant when controlling for age and BMI. The OR calculated were as follows: Triple negative 1.00 (confidence interval [CI]: 0.6-1.666, p = 0.9996), ER+ 1.063 (CI: 0.89-1.226, p = 0.7877), PR+ 1.005 (CI: 0.76-1.506, p = 0.9811), HER2+ 1.011 (CI: 0.78-1.768, p = 0.8535), recurrence: 1.090 (CI: 0.63-1.837, p = 0.7558). There were no significant interactions with DM and T, N stage when controlling for age and BMI. T stage: p=0.692, N stage : p=0.7349 and recurrence p=0.5469 on recurrence controlling for age, BMI and grade: p=0.5462. There was no significant association between grade in DM vs. no DM with rates of grade in DM as follows: grade 1: 10.81%, grade 2: 49.55%, grade 3: 39.64% and in non-DM grade 1: 13.55%, grade 2: 45.8% and grade 3: 40.65%. Mean score difference p=0.7588

**Conclusions:** We did not find significant differences in our DM women compared with non-DM women when evaluating tumor subtype, T stage, N stage, grade or recurrence. In our patient population these factors do not play a role in the increased cause specific mortality in DM patients with breast cancer. Other factors must play a role in this and should be explored further.

**P1-08-25**

**Breast Cancer Burden, Risks and Outcomes in Latin America.**

Justo NA, Wilking NE, Jönsson B. OptumInsight, Stockholm, Sweden; Karolinska Institutet, Stockholm, Sweden; Stockholm School of Economics, Stockholm, Sweden

**BACKGROUND:** Breast cancer (BC) burden and care vary substantially across Latin America (LA). Aim: To provide an overview of the epidemiology, outcomes and countries’ risk profiles in LA to identify opportunities for improvement.

**METHODS:** Review of literature (PubMed, LILACS, SCIELO), public databases (Globocan 2002 & 2008, CEPALSTAT, DIRAC, PAHO, WHO/YS, etc) and conference presentations (ASCO, ISPOR). Local experts & patient organizations were surveyed. Current
Age is the main risk factor; while the association of education and wealth with BC occurrence is less significant. Reproductive and lifestyle-related risk factors, proven at a patient level, were not significant at a country-aggregate level and the specialized literature indicates that the evidence on associations between BC and diet is inconclusive.

### Discussion
LA’s considerable variation embedded within per-country averages may be masking underlying causality. Though, it can be inferred that there is no one-suit-all prevention campaign and policies aimed at reducing the risk of developing BC will succeed as long as they are properly targeted and tailored for different needs. Early diagnosis and population-wide access to treatment following policies aimed at reducing the risk of developing BC will succeed with a minimum 2.0-fold change of expression, 119 probes from 106 genes were differentially expressed, with 35 genes expressed at significantly higher levels and 71 at significantly lower levels in tumors from obese women compared to those from healthy-weight patients. Hierarchical clustering using these 119 probes reliably partitioned all samples as healthy-weight or obese. Genes differentially expressed include lower expression of several members of the histone cluster 1 family, APOD and IGF1, and higher expression of FABP7, members of the major histocompatibility complex and LTF in tumors from obese women.

### Conclusions
Invasive breast tumors from obese women are genetically different from those of healthy-weight women. Altered gene expression may affect tumor cell proliferation and survival, contributing to aggressive phenotypes and altered immune response. These underlying molecular differences may contribute to the less favorable prognosis observed in obese women with breast cancer.

### P1-09-02
**HDL-Cholesterol and Low-Penetration Gene CYP17 rs2486758 Influence Daily Estrogen Levels. The EBBA-I Study.**

**Iversen A, Thune I, McTiernan A, Makar KW, Wilsgaard T, Ellison PT, Jasienska G, Flote V, Poole E, Furberg A-S. University of Tromso, Tromso, Norway; Oslo University Hospital, Oslo, Norway; Fred Hutchinson Cancer Research Center, Seattle, WA; Harvard University, Cambridge, MA; Medical College, Krakow, Poland; Channing Laboratory, Boston, MA; University Hospital of North Norway, Tromso, Norway.**

**Objectives:** The low-penetration gene CYP17 encodes cytochrome P450 enzymes, catalysts in the endogenous estrogen biosynthesis. Low levels of high-density lipoprotein cholesterol (HDL-C) have been associated with increased estrogen level1, increased breast cancer risk and poor breast cancer prognosis2. However, the relationship between CYP17 and metabolic profile including HDL-C levels, and estradiol levels remains unclear. Thus, we hypothesize that gene-environment interactions between eight SNPs on CYP17 and metabolic profile including HDL-cholesterol, may influence the levels of biologically active 17β-estradiol of importance of breast cancer development.

**Material and methods:** The Norwegian Energy Balance and Breast cancer Aspects Study (EBBA-I) includes 203 healthy women (25-35 years) that underwent clinical examinations, blood sampling for metabolic profiling in serum and DNA extraction from whole blood, and daily saliva sampling throughout an entire menstrual cycle. Daily saliva levels of 17b-estradiol were measured by radioimmunoassay at the Reproductive Ecology Laboratory, Harvard University, USA. Tagging SNPs (rs1004467, rs743575, rs4919687, rs37811286, rs3824755, rs10786712, rs743572, rs2486758) representing CYP17 variability in the Caucasian population were selected using MAF > 5% and r2 = 0.80. The polymorphisms were genotyped at the Molecular Epidemiology Laboratory, Fred Hutchinson Cancer Research Center, USA. A clustered metabolic risk score was defined based on WHO criteria for the metabolic syndrome. Multivariable linear and generalized estimating equation regression models were used to study the associations.

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**BC epidemiology and outcomes**

<table>
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<tr>
<th>Country</th>
<th>Incidence ASR 2008</th>
<th>Mortality ASR 2008</th>
<th>Mean age at diagnosis</th>
<th>Stage (% T+II)</th>
<th>MIR 2002</th>
<th>MIR 2008</th>
<th>Change (%)</th>
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<td>0.33</td>
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</table>

**Risk factor Correlation Coefficient**

- Births women under 30: -0.485, 0.131
- Age at childbearing: 0.378, 0.252
- Fertility rate: -0.309, 0.355
- Overweight & obesity (fem): 0.219, 0.518
- Alcohol consumption (fem): 0.112, 0.791
- GDP per Capita: 0.688, 0.019
- Education (fem): 0.607, 0.051
- Mean Age (fem): 0.846, 0.801
Result: Women with at least one variant allele of the CYP17 rs2486758 combined with the highest metabolic risk score (i.e., upper tertile), had 53% higher levels of daily salivary 17β-estradiol throughout the entire menstrual cycle compared with all other women (P < 0.001). Among women in the upper tertile of metabolic risk score, those with the variant allele had 43% higher levels of daily salivary 17β-estradiol (P = 0.002). Similarly, the combination of having the variant allele and serum total cholesterol/HDL-C ratio in the upper tertile was associated with 43% higher levels of daily salivary 17β-estradiol, compared with all other women (P = 0.005), and 34% higher levels of daily salivary 17β-estradiol, compared with the women with common allele in the upper tertile (P = 0.038).

Conclusion: Our results suggest that a polymorphism in CYP17 (rs2486758) may represent a strong genetic predisposition to increased endogenous estrogen levels in women with an unfavourable metabolic profile and a high total cholesterol/HDL-C ratio. Thus, total cholesterol/HDL-C ratio may be a clinical biomarker for breast cancer development in these subsets of women.

References

P1-09-03

Prevalence of Germline TP53 Mutations in Young Women with HER2-Positive Breast Cancer.

Dick MG, Masciari S, Miron A, Miron P, Foley K, Gelman R, Dillon DA, Richardson AL, Verselis SJ, Lypas G, Krop JE, Garber JE. Dana Farber Cancer Institute, Boston, MA; Brigham and Women’s Hospital, Boston, MA

Background: Li Fraumeni syndrome is a rare inherited cancer susceptibility condition associated with germline mutations in the TP53 gene, in which breast cancer (BC) is the most frequent tumor. The prevalence of TP53 mutations in population-based series of very young onset BC (<30 years at diagnosis) ranges from <1% to approximately 7% 

Results: We identified 347 women with invasive HER2 positive BC diagnosed at age < 50 years using the Clinical Operations and Research Information System (CORIS) at the Dana Farber Cancer Institute. Information on age at diagnosis, histology, hormone receptor and HER2 status as well as personal and family cancer history was confirmed from medical records. 129 patients were excluded for various reasons, including a cancer diagnosis prior to the BC and a documented BRCA1/2 mutation. A combination of Exon Grouping Analysis (EGAN) and Sanger sequencing for detection of TP53 mutations in exons 2-11 including surrounding intronic sequence was performed on 218 germline DNA samples. Multiplex Ligation-dependent Probe Amplification (MLPA) analysis for the detection of TP53 deletions or duplications is ongoing.

Results: A germline TP53 mutation was identified in 4 women diagnosed at age < 50 years (1.8%, 95%CI 0.5-4.6). At BC diagnosis, they were 23, 32, 44 and 50 years. Two BC were ER+/PR+, HER2+ and 2 were ER-/PR-, HER2+. Estimate of prevalence of germline TP53 mutations by age at BC diagnosis are: age ≤ 35, 2/41 (4.9%, 95%CI 0.6-16.6), and age ≤ 45 3/168 (1.8%, 95%CI 0.4-5.1). Among the women with germline TP53 mutations, 2 met the Chompret criteria and none the classic LFS criteria.

Discussion: TP53 mutations were identified in a cohort of women with HER2+ BC at young age. As expected, the frequency is higher in younger women, but mutations were seen in all age groups that were evaluated. None of these women met classic LFS criteria by family history. Consideration of TP53 testing should be given to women diagnosed below age 35 who are negative for BRCA1/2 mutations regardless of family history. Analysis of other series will be helpful in reaching more stable estimates of the prevalence of mutation carriers among patients with HER2+ BC at young age.

7. Masciari S et al: J Clin Oncol 29; 2011 (suppl; abstr 1519)

P1-09-04


Sueta A, Ito H, Iwata H, Hosono S, Watanabe M, Iwase H, Tajima K, Tanaka H, Matsuo K. Aichi Cancer Center Research, Nagoya, Japan; Kumamoto University Graduate School of Medical Science, Kumamoto, Japan; Aichi Cancer Center Central Hospital, Nagoya, Japan; Nagoya University Graduate School of Medicine, Nagoya, Japan

Objective Genome-wide association studies (GWASs) have identified genetic variants associated with breast cancer. Most GWASs to data were conducted in women with European background and the extent to which these variants contribute as predictors of breast cancer among Japanese population is unknown.

Methods We analyzed 24 genetic variants that have been identified in previous GWASs and conducted a case-control study with 697 cases and age- and menopausal status- matched 1394 controls in the framework of the Hospital-based Epidemiologic Study at Aichi Cancer Center (HERPACC). All subjects were asked to provide information on lifestyle factors and blood samples for genetic studies. We fit conditional regression models with genetic variants and conventional risk factors including age, age at menarche, menopausal status, current body-mass-index, age at first live birth, regular exercise, family history of breast cancer, and referral pattern to our hospital. In addition, we created a polygenetic risk score, using the single nucleotide polymorphisms (SNPs) with statistically significant association with the breast cancer risk to measure the cumulative effect of multiple genetic risk variants. Furthermore, we evaluated the prediction model that included conventional risk factors by comparing with and without the genetic risk score, using c statistic.

Results Eleven SNPs (FGFR2-rs2981579, rs2981578, rs1219648, rs2402094, rs2981582, TOX3/TNRC9-rs8051542, rs3803662, LOC643714-rs4784227, C6orf97-rs2046210, 8q24-rs13281615, SLC4A7-rs4973768) revealed significant associations with breast cancer among Japanese population is unknown.

Discussion Among women with at least one variant allele of the CYP17 rs2486758 combined with the highest metabolic risk score (i.e., upper tertile), had 53% higher levels of daily salivary 17β-estradiol throughout the entire menstrual cycle compared with all other women (P < 0.001). Among women in the upper tertile of metabolic risk score, those with the variant allele had 43% higher levels of daily salivary 17β-estradiol (P = 0.002). Similarly, the combination of having the variant allele and serum total cholesterol/HDL-C ratio in the upper tertile was associated with 43% higher levels of daily salivary 17β-estradiol, compared with all other women (P = 0.005), and 34% higher levels of daily salivary 17β-estradiol, compared with the women with common allele in the upper tertile (P = 0.038).

Conclusion: Our results suggest that a polymorphism in CYP17 (rs2486758) may represent a strong genetic predisposition to increased endogenous estrogen levels in women with an unfavourable metabolic profile and a high total cholesterol/HDL-C ratio. Thus, total cholesterol/HDL-C ratio may be a clinical biomarker for breast cancer development in these subsets of women.

References
women with scores of 4-5, 6-7, 8-9 and 10 or more were 1.33 (95% confidence interval, 1.00 - 1.80), 1.71 (1.26 - 2.30), 3.01 (1.97 - 4.58) and 8.69 (2.74 - 27.5), respectively (P<0.001). The ORs for premenopausal women with the corresponding risk scores were 1.71 (1.12 - 2.63), 1.79 (1.15 - 2.78), 3.70 (1.98 - 6.93), and 14.0 (3.30 - 59.5), respectively, and those for postmenopausal women with the corresponding risk scores were 1.09 (0.72 - 1.66), 1.71 (1.12 - 2.61), 2.60 (1.44 - 4.71), 3.75 (0.57 - 24.4), respectively, compared to those with scores of 3 or less (each P<0.001). The c statistic for a model including the genetic risk score in addition to the conventional risk factors was 0.633, whereas 0.602 without it (P<0.001). Population-attributable fraction of the risk score was 33.8%.

**Conclusion** we identified a genetic predictor of breast cancer in a Japanese population. A risk model including genetic risk score may be useful to distinguish women at high-risk of breast cancer from those at low-risk, particularly in the context of targeted prevention.

**PI-09-05**

**A 3'UTR Functional Variant in BRCA1: A Predictor of Poor Outcome in Breast Cancer.**

Dorairaj JJ, Miller N, Newell J, Kerin MJ, Weidhaas JB. National University of Ireland, Galway, Ireland; Yale University, New Haven, CT

**Background:** MicroRNAs (miRNAs) are class of gene regulators which exert their effects through binding with partial complementarity to sequences in the 3' untranslated region (3'UTR) of the target mRNA. Single nucleotide polymorphisms (SNPs) in the 3'UTR of target mRNA have the potential to disrupt or create new illegitimate miRNA targets and have been associated with cancer predisposition as well as tumor biology. An association between rs8176318 and risk for familial breast and ovarian cancer as well as risk for triple negative breast cancer in African American women has previously been established. We aimed to evaluate the phenotypic effect of rs8176318 in a west of Ireland breast cancer cohort, and also its role as a biomarker of prognosis.

**Methods:** DNA from 727 unselected breast cancer cases and 387 controls were extracted from whole blood and genotyped for rs8176318 in the 3'UTR of the BRCA1 oncogene. The association with disease specific parameters and outcome were evaluated.

**Results:** Overall, there was a significant difference in the distribution of the three genotypes between cases and controls (p=0.035). The dominant variant model was predictive of breast cancer (OR=1.4, 95% CI 1.1-1.8). Fifty-two percent of breast cancer cases had the variant, with similar prevalence between subtypes: Luminal A (279 [54%] of 519 cases), Luminal B (37 [43%] of 85 cases), HER2 (21 [53%] of 40 cases) and triple negative breast cancer (41 [49%] of 83 cases). Comparing the prevalence of the variant within respective subtypes with controls however, Luminal A breast cancer was most strongly associated with rs8176318 (OR=1.5, 95%CI 1.1-1.9). The variant was not significantly associated with disease free survival (DFS) in all cancer cases (Log-rank test=0.084). However, rs8176318 was predictive of a poorer DFS (Log-rank test=0.041) in Luminal A patients with a 10-year DFS of 54% (95% CI 0.4-0.7) and 77% (95% CI 0.7-0.9) for the variant versus the non-variant, respectively. In addition, patients with Stage IV disease had a 6-fold increased risk of carrying the variant (p=0.035), with 17 (73%) of 23 patients with metastasis at presentation positive for the variant compared to 324 (50%) of 642 patients without metastasis (p=0.034). Luminal A patients with Stage IV disease had a 13-fold risk of carrying the variant (p=0.043) in regression analysis, controlling for all other clinicopathological variables. Similarly, the variant was associated with distant metastasis in Luminal A patients, with 11 (92%) of 12 patients with metastasis positive for the variant compared to 242 (52%) of 463 patients without metastasis (p=0.028).

**Conclusion:** These findings suggest that rs8176318, a variant in the 3'UTR of BRCA1, is a genetic marker for modest breast cancer risk but aggressive tumor biology in breast cancer, and highlights the need for further clinical and biological evaluation of such markers.

**PI-09-06**

**Single Nucleotide Polymorphisms in the BRMS1 and SIPA1 Metastasis Suppressor Genes as Prognostic Markers in Breast Cancer Patients.**

Roberts MR, Hong C-C, Edge SB, Yao S, Nesline M, Ambrosone CB. Roswell Park Cancer Institute, Buffalo, NY

**Introduction:** Single nucleotide polymorphisms (SNPs) in the metastasis suppressors BRMS1 and SIPA1 may affect metastatic efficiency. BRMS1 affects apoptosis, colonization, cell adhesion, and invasive potential. Loss of BRMS1 expression has been correlated with younger age at diagnosis and reduced survival time in patients with progesterone-receptor (PR) negative, HER2-positive tumors. SIPA1 affects extracellular matrix gene expression and cell adhesion, and while SNPs have been associated with node positive, estrogen-receptor (ER)/PR negative tumors, evidence for a relationship with survival has been conflicting. Identifying SNPs that affect risk of recurrence and survival may improve the ascertainment of patients who require aggressive adjuvant therapy following a diagnosis of breast cancer. We evaluated associations between seven BRMS1 and SIPA1 SNPs and recurrence and survival in patients with primary breast cancer.

**Methods:** We identified 1,015 incident breast cancer patients who received surgery at Roswell Park Cancer Institute (RPCI) and participated in the DataBank and BioRepository (DBBR) resource. Participants completed an epidemiologic questionnaire and provided a blood sample prior to surgery or other treatment. Clinical and pathologic data were linked to de-identified participant data in the DBBR database. SNPs in BRMS1 (rs11537993, rs3116068, and rs1052566) and SIPA1 (rs75894763, rs746429, rs3741378, and rs2306364) were genotyped by RPCI's Genomics facility using Sequenom® iPLEX Gold and Taqman® real-time PCR assays. Cox proportional hazards regression was used to estimate hazard ratios and 95% confidence intervals.

**Results:** The median follow-up time was 33 months, and 49 deaths and 42 recurrences occurred. Tumors were more likely to be larger, node positive, ER/PR negative, and high grade among patients who experienced a recurrence or death. Recurrence was less likely in older patients and those with higher body mass index, although the latter association was nonsignificant (p=0.06). Patients with at least one variant allele of the BRMS1 rs3116068 genotype experienced shorter overall survival compared to patients with the homozygous common genotype (HR=2.05, 95% CI 1.15-3.63, rs3116068 AG+AA compared to GG). The remaining SNPs were not associated with overall survival, and none of the SNPs were associated with recurrence.

**Conclusions:** In our data, the variant allele of rs3116068 was more common among women whose breast cancer was node negative and HER2-positive, compared to those with the common rs3116068 allele. The rs3116068 variant allele is also associated with poorer survival. While our findings do not support a role for common SNPs in the SIPA1 gene in breast cancer prognosis, BRMS1 rs3116068 may be a useful prognostic biomarker. Future goals are to examine additional SNPs in BRMS1 and other metastasis-related genes in a larger, racially diverse population.
Funding: This research was supported by a gift from the Jayne and Phil Hubbell Family. The DBBR and Genomics Facility are RPCI Cancer Center Support Grant shared resources, supported by P30 CA016056-32. Ms. Roberts is a DOD Predoctoral Award recipient (BC10068) and Dr. Ambrosone is funded by the Breast Cancer Research Foundation.

P1-09-07

Background: The exact contribution of TP53 germline mutations, associated with Li Fraumeni Syndrome - LFS, to the overall burden of cancer is still only partially known. Studies in Southern and Southeastern Brazil have shown that a particular mutant, TP53 p.R337H, has incomplete penetrance and may be present in a significant number of subjects (estimated frequency at the populational level of 1:300 individuals). In an exploratory approach, the aim of this study is to assess the prevalence of the TP53 p.R337H mutation in women with breast cancer diagnosed before 46 and after 55 years of age, unsellected for family history of cancer and resident in Southern and Southeastern Brazil.

Methods: Formalin-fixed paraffin-embedded (FFPE) non-tumoral tissue of 521 women diagnosed with breast cancer between 2000 and 2010 in two pathology laboratories were obtained retrospectively and consecutively, and analyzed after anonymization. Genomic DNA was isolated with the QIAamp DNA Tissue Kit and genotyping performed by allelic discrimination using a TaqMan assay. Confirmation of all mutation-positive and a sample of mutation-negative cases was done by TP53 exon 10 sequencing.

Results: Analysis of the first 299 cases identified the TP53 p.R337H mutation in the germline of 15 (5.0%) cases: 13/142 (9.2%) before 46 years and 2/157 (1.3%) diagnosed after 55 years. The p53 expression pattern assessed by immunohistochemistry in the breast tumors was not different between p.R337H mutation carriers and non-carriers.

Conclusion: Preliminary analysis in a sample of women with breast cancer diagnosed before 46 and after 55 years of age, unsellected for family history of cancer and resident in Southern and Southeastern Brazil indicates that the germline TP53 p.R337H mutation-positive and a sample of mutation-negative cases was done by TP53 exon 10 sequencing.

P1-09-08
Association of Hypoxia-Inducible Factor-1 with Breast Cancer Risk: A Meta-Analysis of Published Studies.
Yin W, Liu G, Lu J, Shen Z, Shao Z. Fudan University Shanghai Cancer, Shanghai, China

Background: Hypoxia-inducible factor-1 (HIF-1) plays an important role in the development and progression of breast cancer. However, conflicting results have been yielded for the association between HIF-1 polymorphisms and breast cancer risk. Therefore, we carried out a meta-analysis to assess the association of HIF-1 1772 C/T and 1790 G/A polymorphisms with breast cancer.

Material and methods: Computerized and manual searches were performed to identify eligible studies comparing allelic and genotypic frequency between cases and controls in breast cancer. Odds ratios (ORs) were used to estimate the association between HIF-1 polymorphisms and breast cancer risk. The fixed-effects or random-effects model was used to combine data. Subgroup analyses by ethnicity were also performed.

Results: This meta-analysis for 1772 C/T polymorphism included 1934 breast cancer cases and 1848 controls, and for 1790 G/A polymorphism included 602 breast cancer cases and 479 controls. We found that 1772C/T polymorphism increased the risk of breast cancer in the recessive model (OR = 2.273, 95% CI: 1.061-4.872, P = 0.035), whereas similar effect failed to be observed in the dominant model (OR = 1.075, 95% CI: 0.717-1.613, P = 0.725). There was a significant increase in breast cancer risk among Asian women (OR = 4.162, 95% CI: 1.508-11.484, P = 0.006) but not Turkish patients in the recessive model. For the 1790G/A polymorphism, we found that it had no exact effect on the decreased risk of breast cancer in both recessive and dominant model.

Discussion: This meta-analysis indicates that the polymorphism of HIF-1 1772C/T rather than 1790G/A polymorphism might be one of the high risk factors for breast cancer. The effect of the 1772C/T polymorphism on breast cancer risk is completely generated by the Asian women.

P1-09-09
Fetal Microchimerism and In Situ Breast Cancer.
Eun JK, Gadi VK. University of Washington, Seattle, WA; Fred Hutchinson Cancer Research Center, Seattle, WA

Background: Haploidentical fetal cells acquired and maintained by a woman in her blood and tissues long after pregnancy completion, also known as fetal microchimerism, is inversely associated with invasive breast cancer. Furthermore, in healthy women, absence of fetal microchimerism serves as a risk factor for development of future breast cancer (J Clin Oncol 29: 2011 suppl; abstr 1574). Here, we test the hypothesis that failure to maintain fetal microchimerism following pregnancy is also associated with in situ breast cancer.

Methods: Buffy coat specimens were obtained from the Roswell Park Cancer Institute Data Bank and BioRepository from parous patients with in situ breast cancer and parous control volunteers with no prior history of cancer. Using quantitative PCR, DNA extracts from peripheral blood buffy coat specimens were tested by a single female operator blinded to case:control status for the presence (and concentrations) of Y chromosome sequence DYS14, a marker of male fetal microchimerism.

Results: In this interim analysis, 83% (34/41) of healthy control women and 55% (29/53) in situ breast cancer patients tested positively for the presence of DYS14. The unadjusted odds ratio was 0.25 (95%-CI of 0.08-0.72; p=0.008). Median concentrations were also
greater in healthy women compared to women with in situ cancer, 0.15 versus 0.02 fetal cell equivalents per 10^5 proband genomes, respectively (p=0.004).

Summary: Preliminary results suggest that the associations of fetal microchimerism and in situ breast cancer are similar in magnitude and direction to that observed for invasive disease. The natural history of in situ breast cancer is for a large portion of patients to develop invasive disease in 8–10 years absent of comprehensive locoregional control; results here point to a failure to maintain or loss of haploidentical fetal cells well before development of invasive disease. Final results from the entire cohort planned (n=200) with adjustment for anthropometric, reproductive, and cancer specific factors will be presented at the meeting.

P1-10-01
Utility of Routine Cardiac Ejection Fraction (CEF) Measurement Prior to Anthracycline-Based Chemotherapy (ABC): A Study of 466 Patients with Early-Stage Her2-Negative Invasive Breast Cancer.

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Background: Despite the lack of evidence to support its utility, routine CEF measurement prior to ABC is a common practice in the community, required in most US clinical trials, endorsed by the American Heart Association and American College of Cardiology, and appears on Food Drug Administration-approved labeling guidelines.

Objectives: We determined the frequency of the following events in newly diagnosed invasive breast cancer patients: a) CEF measurement prior to ABC; b) asymptomatic left ventricular dysfunction; c) modification in initial treatment strategy as a result of CEF measurement; d) development of congestive heart failure (CHF) after ABC.

Methods: From our cancer registry, we obtained a list of all female patients with newly diagnosed stage I-III breast cancer who were treated with chemotherapy as part of initial therapy at our institution in 2000-2010 (N=562). We excluded those with prior CHF, ABC, and those who had Her2-positive disease (n=66). CEF <50% was considered low. CHF risk factors including coronary heart disease, cigarette smoking, hypertension, obesity, and diabetes mellitus were collected.

Results: We included 496 patients with the following stage distribution: I (22%), II (54%), and III (24%). The mean age was 53 years (SD±11; range 25-88). In 30 (7%) patients, ABC was considered inappropriate by the physician provider due to poor performance status, co-morbidities, and physician or patient preference. Of the 466 patients considered for ABC, CEF was measured in 241 (52%) patients before chemotherapy. In the latter group, only 1 (0.4%) patient was found to have asymptomatic left ventricular dysfunction with CEF of 48%. Among patients considered for ABC, 10 (2%) did not receive ABC for the following reasons: low or borderline CEF (2), patient preference (3), co-morbidities (2), and randomization to non-ABC in clinical trials (3). After a mean follow up 57 months (SD±34), only 3 of 456 (0.7%) patients who received ABC developed CHF. Two had normal CEF prior to treatment while the other did not have CEF measured. The practice of CEF measurement prior to ABC varied among physician providers, ranging from 23% to 67% (mean/SD 47%/±15%). Those who had CEF measured before chemotherapy were older (55 vs. 50 years; P<0.001) and had more CHF risk factors (1.3 vs. 0.9; P<0.001).

Conclusions: At our institution, routine CEF measurement prior to ABC as initial therapy for early-stage Her2-negative invasive breast cancer patients is a common though not uniform practice. Asymptomatic left ventricular dysfunction is rarely detected and generally mild. Our findings do not support the utility of routine CEF measurement in this population and challenge current practice and guidelines. Potential cost implications to our health care system can be substantial.

P1-10-02

Baffert S, Cottu PH, Kirova Y, Bachelot T, Le Rhun E, Mercier F, Levy C, Gutierrez M, Madrange N, Moldovan C, Guis S, Serin D, Cotte FE, Benjamin L, Simondi C, Maillard C, Laulhere-Vigneau S, Durand-Zaleski I, Institut Curie, Paris, France; Centre Léon Bérard, Lyon, France; Centre Oscar Lambret, Lille, France; Stat Process, Port Mort, France; Centre François Baclesse, Caen, France; Institut Curie, Saint-Cloud, France; Institut Bergonie, Bordeaux, France; Centre Henri Becquerel, Rouen, France; Centre GF Leclerc, Dijon, France; Institut Sainte Catherine, Avignon, France; GlazoSmithKline, Marly Le Roi, France; Ceri Medical, Garches, France; CHU Henri Mondor, Créteil, France

Background
HER2+ status is associated with poor prognosis and a high incidence of brain metastases (BM) in breast cancer (BC). Addition of HER2-targeted therapies to conventional chemotherapy has significantly improved survival in HER2+ patients (pts). Management of BM implies a multidisciplinary therapeutic approach involving medical oncology, radiation oncology and neurosurgery teams. Nevertheless available data evaluating health resources use and associated costs are limited. Our objective was to describe treatment patterns and healthcare costs associated with HER2+ BMBC patients.

Patients and methods
An observational retrospective study was conducted on 207 HER2+ BC pts, newly diagnosed with BM as first site of relapse or as secondary metastases between January 2006 and December 2008. Pts were recruited in 10 hospitals, all funded by a prospective payment system based on Diagnosis Related Groups (DRG). Individual data concerning initial diagnosis, distant and BM relapses, treatments, complications and hospitalization stays were collected during a 2 year - follow-up. DRGs 2007 official tariffs (per-case payment basis) and 2007 expensive innovative drugs tariffs (drugs paid to hospitals by Health Insurance in addition to per-case payments) were used to estimate direct medical costs from the Health Insurance perspective. Survival was estimated using Kaplan Meier method. In the presence of cost-censored data, a partitioned estimator was used to adjust censoring costs (Bang and Tsiatis, Biometrics, 2002).

Results
91.8% (190/207) of BMBC pts received radiation therapy, 84.5% (175/207) received chemotherapy including HER2 targeted treatments and 12.6% (26/207) were treated by neurosurgery. 72.5% (150/207) of pts were hospitalized at least once during the follow-up period. Pts had on average 2.90 hospital stays (range 1-8). The median duration of stay was 9 days (1-221). Complications leading to re-hospitalization were recorded in 45.9% (95/207) of pts. The median overall survival from the diagnosis of BM was 13 m. Hospital healthcare costs were concentrated on the 6 first months following BM diagnosis. Mean cost of BMBC management was 18,480€/patient within the 6 first months and decreased to 16,306€ from 7-12 months, 15,844€ from
13-18 months, and 15,225€ from 19-24 months. The proportion of costs attributed to inpatient hospitalizations (treatments and complications) was similar to the one attributed to drugs whatever the period of follow-up. Pts with BM as first site of relapse consumed more healthcare resources compared to pts with secondary BM (38,813€/pt vs 32,253€/pt after one year of follow-up, respectively).

Conclusions
Healthcare resources spending is mainly concentrated at the beginning of metastatic disease management, especially for patients with BM as first site of relapse. These results illustrate the use of expensive treatments in the first months following BM diagnosis. Individual data derived from this observational study allowed us to gather more specifically treatment patterns for HER2+ BMBC in order to estimate the costs more accurately.

P1-10-03
Colon Stimulating Factor Use with Taxane-Based Therapy for Metastatic Breast Cancer: Claims Analysis of Prophylaxis, Treatment, and Costs.
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Background: The taxanes (paclitaxel, docetaxel, and albumin-bound paclitaxel) are used in women with metastatic breast cancer (MBC). Clinical trials indicate that these drugs may differ in their toxicity profiles, including rates of neutropenia and need for colony stimulating factor (CSF) supportive therapy. Utilizing medical claims data, we evaluated prophylactic and treatment-related CSF use among women receive taxane-based chemotherapy regimens.

Objective: To determine if differences exist in rates of CSF use for prophylaxis, treatment and associated costs in women receiving taxane-based chemotherapy for MBC.

Methods: Women with MBC were identified with ICD-9-CM codes and by their prior use of adjuvant chemo regimens. Paid medical claims (source: Ingenix Consulting) from May 1, 2006 to April 30, 2009 were analyzed. Study groups were defined according to the first taxane administered. CSF utilization was classified as prophylaxis (0-5 days post-taxane administration), treatment (6-21 days post-taxane,) or non-taxane associated use (>21 days post-taxane). Comparisons were made between the taxane groups. All CSF costs were captured from date of first taxane to end of taxane therapy +21 days and categorized as above. Patients censored if ≥35 days elapsed with no taxane therapy. Two-stage regression analyses were performed. Control variables included age, Romano comorbidity score, and use of prior and other concurrent chemotherapy.

Results: 4,503 women (mean age: 53 +/- 10 years) with MBC were identified: 2,599 in the docetaxel group; 1,643 received paclitaxel; and 261 received nab-paclitaxel. More patients receiving docetaxel received any CSF (75.6%) than either paclitaxel (49.8%) or albumin-bound paclitaxel (36.8%) P<0.05 for each comparison. For docetaxel 70.5% of women received prophylactic CSF, whereas the rates were 47.0% for paclitaxel and 33.0 for albumin-bound paclitaxel (P<0.05).

The rate of women receiving CSF in the albumin-bound paclitaxel (36.8% P<0.05 for each comparison). For docetaxel 70.5% of women received prophylactic CSF, whereas the rates were 47.0% for paclitaxel and 33.0 for albumin-bound paclitaxel (P<0.05).

Conclusions: Compared with docetaxel and paclitaxel, patients receiving albumin-bound paclitaxel had significantly lower prophylactic CSF use, yet did not experience any difference in treatment-related use. Daily per-patient CSF expenditures for albumin-bound paclitaxel were significantly lower than the other two taxanes.

P1-10-04
Cost-Utility of the 21-Gene Breast Cancer Assay (Oncotype DX®) in the Irish Healthcare Setting
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Objective: The Oncotype DX® Breast Cancer Test is a validated 21-gene assay that predicts the likelihood of benefit from adjuvant chemotherapy in early-stage, node-negative and up to 3-node-positive, ER+ breast cancer as well as the likelihood of distant recurrence at 10-years. Study objective was to investigate the cost-utility (incremental cost per QALY gained) of using Oncotype DX® in early stage, node-negative, ER+ breast cancer patients, from the perspective of the Irish Healthcare system.

Methods: A cost-utility analysis was performed over the lifetime of the patient using a state transition model (Markov), which simulated the costs and quality-adjusted survival associated with and without the use of Oncotype DX® to inform adjuvant chemotherapy decisions. The model included 3 health states: no recurrence, recurrence and death. Transition probabilities between health states, utility scores associated with each of the health state, patient cohort characteristics and impact of Oncotype DX® on chemotherapy decisions data were derived from published sources. Direct medical costs associated with chemotherapy were collected from a representative panel of 5 hospitals in Ireland. Costs and outcomes were discounted at 4% per annum and the analysis was conducted over 30 years (lifetime horizon). Probabilistic and one-way sensitivity analyses were conducted.

Results: Costs associated with adjuvant chemotherapy (4 cycles of TC) included the following costs: drug (€1,002); administration and monitoring (€1,646); adverse event prevention (€3,561) and adverse event management (€756). The average total cost of chemotherapy across hospitals therefore summed up to €6,965. The base case incremental cost per QALY gained with ODX testing was estimated to be €9,462. There was a 74% chance for ODX to be cost-effective at a willingness to pay threshold of €20,000.

Conclusions: Using Oncotype DX® as a decision tool to inform adjuvant chemotherapy in node-negative, ER+ breast cancer patients, is likely to be cost-effective in the Irish healthcare setting. This estimate is probably conservative as the cost and disutility associated with long-term impact of chemotherapy related adverse events (i.e. such as cardiotoxicity, secondary leukaemia and potential cognitive impairment) have not been considered.

P1-10-05
Is the 21-Gene Breast Cancer Test (Oncotype DX®) Cost-Effective?
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Background: The Oncotype DX® Breast Cancer Test is a validated 21-gene assay that predicts 10 year risk of recurrence and the likelihood of benefit from adjuvant chemotherapy in early-stage, node-negative ER+ breast cancer. The cost-effectiveness of using Oncotype DX® has been published in several countries but to date, there hasn’t been any review of these studies.

Materials and methods: The electronic database Pubmed and a selection of congress databases were searched using combinations
of search terms designed to identify publications describing cost-effectiveness analyses of Oncotype DX® in early stage breast cancer patients. Searches were limited to those published in the English language between January 2001 and April 2011. All records were screened for inclusion in the review. The methodological quality of selected publications was assessed using the 35 items methodological checklist from Drummond et al (1996).

Results: Five published health economics analyses and 3 abstracts (two posters and an oral presentation) were identified. The studies were carried out in several countries (US (2), Canada (2), Japan, Israel, Singapore and Hungary and have used a Markov modelling approach based on data from a large multicentre trial (e.g. NSABP B-20) to make estimates of long-term outcomes, and assess the cost-effectiveness of using the Oncotype DX® recurrence score in patients classified as having a high or low risk of distant recurrence using other methods of assessment. All studies were carried out in the perspective of the healthcare payer, and therefore did not consider broader costs to the patients and society. Study comparators, costs, characteristics of the population receiving the test and impact of using the Oncotype DX® results on treatment decisions were adapted to each individual country clinical practice explaining the large range of cost-effectiveness results from these studies. In the US, using Oncotype DX® was shown to be cost-saving when in one of the Canadian studies, it was likely to be cost-effective (incremental cost-effectiveness ratio of $64,063 per QALY gained). Consistently across all five studies, use of Oncotype DX® was projected to improve survival (where reported), quality-adjusted life expectancy and to reduce chemotherapy costs versus comparators. When looking at the methodological quality of studies, they generally scored well with positive responses to 24 or more of the 35 questions on reporting. The exception was the Lyman et al. (US) paper where only 17 positive responses were recorded. The two posters, as expected scored lower than the full scale articles with positive responses of 15 and 18 out of 35 items.

Conclusions: Published literature to date is of good methodological quality and consistently supports the cost-effectiveness of using Oncotype DX® in the various settings. Further analyses should be carried on to assess the budget impact of funding Oncotype DX® and to include a broader perspective of the costs.

P1-10-06
Economic Analysis of Chemotherapy Costs for Adjuvant Therapy in Breast Cancer in France.

Background: Total costs of adjuvant chemotherapy can be estimated using different perspectives. To date, only few studies are available in France and only few of these studies have incorporated all the relevant cost items. Indeed the total cost of adjuvant chemotherapy for breast cancer should include not only the drug costs and their administration but also supportive care, transportation and part of the work absenteeism, because all these costs are borne by the French social security. The study objective was to estimate the total costs of adjuvant chemotherapy in France using two different perspectives: the French social security and society.

Methods: We conducted a retrospective study to calculate the total cost of first line adjuvant chemotherapy for breast cancer in France. We developed an electronic CRF to collect clinical data, chemotherapy drug details, side effects and personal data such as the type of transportation from home to hospital for chemotherapy treatments and duration of work absenteeism. We added the cost of medical consultations, radiology and biology. We also calculated the exact cost of paramedical time and material. All data were collected after patient’s acceptance from clinical records and by phone. Medical resource data were collected from patients’ files for which data were recorded in February 2010 in Tenon hospital (Paris). Unit costs were collected from the French medical insurance database, and other public sources such as national statistics and the technical agency for hospitalization information.

Results: We collected data from 30 patients who had adjuvant chemotherapy for breast cancer. Median age was 57.7 years and 37.9% of patients had a regular work. Using the social security perspective, the mean cost (+/- SD) for pre chemotherapy exams and management (biology, oncologist consultation, implantable port system) was €320 +/-€32. For each chemotherapy cycle, the costs of chemotherapy drugs, preventive medications and chemotherapy administration were €1267 +/-€1424. The cost of chemotherapy adverse events was €405 +/- €829 and €39 +/-€28 for usual monitoring of chemotherapy (biology tests and medical consultations). Transportation costs were estimated at €11 +/-€12 and sick leave payments at €445 +/-€521. The mean total cost per cycle was €1806 +/- €1226 per chemotherapy cycle and €12724 +/- €8426 for the whole adjuvant chemotherapy regimen. Using a broader societal perspective, the total cost of chemotherapy was €14668 +/- €9707 per patient, as it included the full cost of lost productivity due to work absenteeism.

Conclusion: We reported the first cost analysis of adjuvant chemotherapy for breast cancer in France using two different perspectives (the French social security and the society). Using the social security perspective, chemotherapy drugs and their administration accounted for 70% of the total cost of chemotherapy against 60% when using the societal perspective.

P1-10-07
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Background: Despite advances in diagnosis and treatment of breast cancer in the United States, racial disparities in survival persist. Female breast cancer represented the costliest cancer site in 2010 with further increases projected to 2020. Mammography screening guidelines reflect the inherent difficulty in reconciling mortality reduction with potential harms to patients and cost-effectiveness concerns. Contrary still surrounds the 2009 updated USPSTF recommendations, as critics and advocates alike evaluate lives saved, costs, and how best to compare screening strategies. This study simulates USPSTF and ACS guidelines’ effects on stage, 5-year survival, and cost of treatment vs. usual practice in women treated in an urban public hospital.

Methods: Charts of 84 patients diagnosed with Stage I-III breast cancer in 2008 were reviewed. Published tumor doubling times guided a retrospective model to predict size at diagnosis by simulated ACS or USPSTF guidelines. AJCC-7 stages were then assigned to produce 3 distributions: 1) actual stage; 2) stage under ACS; and 3) stage under USPSTF. Survival estimates by stage and year from diagnosis were drawn from NCDB statistics and applied to each stage distribution, yielding average predicted survival for the actual and hypothetical scenarios. Finally, treatment costs for women continuously enrolled in Medicaid for 18 months were calculated from merged claims and registry data and similarly applied.

Results: Study patients averaged 55 years; 85% were African American. Forty-nine percent were covered by Medicaid and 23%
Racial/Ethnic Differences in Adjuvant Trastuzumab Receipt for Women with Breast Cancer within the National Comprehensive Cancer Network.

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Background
Racial/ethnic disparities in breast cancer care are well documented. Although adjuvant trastuzumab has been shown to improve disease outcomes for women with Human Epidermal Growth Factor Receptor 2 (HER2)-positive cancers, the ‘real world’ utilization and toxicity of adjuvant trastuzumab are unknown. Because therapy involves one year of treatment and the costs of treatment are high, a risk for treatment disparity exists. We examined differences in receipt and completion of adjuvant trastuzumab by race/ethnicity, education, employment, and insurance for women diagnosed with HER2-positive breast cancer.

Methods
Using the National Comprehensive Cancer Network (NCCN) Breast Cancer Outcomes Database, we identified 1,146 women with stage I-III HER-2 positive breast cancer who presented to participating NCCN institutions during 2005-2008. In multivariable logistic analyses, we assessed the effect of race/ethnicity on the likelihood of trastuzumab therapy, and among women who initiated trastuzumab, the likelihood of completing ≥ 270 days of therapy, adjusting for center, diagnosis year, age, insurance, comorbidity, education, employment, and tumor characteristics. We also examined reasons for discontinuation of therapy among those who stopped treatment prematurely.

Results
Among patients eligible for this analysis, 75% women were Caucasian, 9% were African-American, and 9% were Hispanic. Most women had managed care insurance (71%) and were employed/student (52%). About one-third (36%) had a college degree and 39% had a high school education or less. Overall, most women (82%) received neo/adjuvant trastuzumab and there were no racial/ethnic
differences in receipt of therapy (adjusted odds ratio [OR] 1.11, 95% confidence interval [CI] 1.72-1.71 for African-American and OR 1.39, 95% CI 1.76-2.54 for Hispanic, versus Caucasian women). Among the 769 women who initiated neo/adjuvant trastuzumab and had ≥365 days of follow-up, 84% completed ≥270 days of trastuzumab. Rates of completion were lower for African-American (72%) and Hispanic (82%) women than Caucasian women (85%). In adjusted analyses, African-American women but not Hispanic women had lower odds of completing therapy compared with Caucasian women (OR 0.45, 95% CI 0.29-0.70, p=0.0003). Indemnity insurance (versus managed care) was associated with lower odds of trastuzumab completion, as was having a high school education or less (versus college education). Among the 123 women who did not complete trastuzumab, 26% stopped early for toxicity, and this occurred more frequently for African-American women than Caucasian women (50% vs. 21%), but small sample precluded a meaningful test for statistical significance.

Conclusion
Compared with Caucasian women, African-American women had similar rates of initiation of adjuvant trastuzumab but much lower rates of completion that were not explained by differences in education, employment, or insurance. Because of the significant benefits conferred by adjuvant trastuzumab therapy for HER2-positive breast cancer, interventions to assure completion of therapy could lead to improved outcomes. Further exploration of racial differences in toxicity and tolerance of therapy are also warranted.

P1-11-03
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Introduction: For women with early stage breast cancer, it is accepted that BCS followed by radiation therapy yields equivalent results to mastectomy. Over the past 20 years, rates of women choosing BCS have increased. However, the national mastectomy rate remains high. Multiple influential factors have been investigated in efforts to define those that aid in surgical decision making, including age, race, education concerning surgical options, and the influence of their surgeon and discussion regarding surgical options. Our study centered on our rural breast cancer population, anticipating that a woman’s surgical choice might be affected by unique rural factors not present in an urban setting. Methods: Stage 0-II breast cancer patients were identified using the Tumor Registry for 1/2000 through 12/2008. Patients were divided into two groups based on surgical procedure, BCS or mastectomy, and mailed a survey. Questions included basic demographics in addition to concerns of recurrence, driving distance to the nearest radiation center, potential need for another operation, influence by educational materials or family members, discussion of options with their surgeon, as well as if they sought a second opinion. Responses were assigned values using a Likert scale. Univariate analyses were performed to detect relationships between surgery choice and survey variables. Those variables found to be significant at the univariate level were entered into a multivariate logistic regression model predicting the probability of mastectomy versus BCS. Results: An overall response rate of 83.1% was achieved, with a total of 283 completed surveys. The mean age at the time of surgery was 61.1 years (SD 9.3), which was not statistically significant. The univariate analyses detected four statistically significant variables related to surgical choice – the worry about recurrence (91.6% mastectomy patients, 55.4% BCS patients: p-value <0.0001), a faster return to “normal life” (51.7% mastectomy patients, 69.9% of BSC patients: p-value 0.039), avoiding another operation (68.9% mastectomy patients, 45.2% BCS patients: p-value 0.012), and having obtained a second opinion (30.5% of mastectomy patients, 16.7% BCS patients: p-value 0.036). There was no difference in surgical choice when comparing for age, level of education, distance from a radiation center, or concerns about driving in inclement weather. The resulting multivariate logistic regression model showed the strongest independent predictor of surgical choice to be worry about breast cancer recurrence, with a p-value of 0.0005 and an odds ratio of 6.1 (95% CI 2.2-16.7). The desire for a faster return to normal life was also significantly predictive in this model. Regarding the patient’s impression of the discussion with their surgeon about their surgical options, 95.2% of mastectomy patients and 89.2% BCS patients stated that their surgeon discussed both as surgical choices. Conclusions: Anticipated rural-unique factors were not related to surgical decision making in our population of early stage breast cancer patients. Concern about breast cancer recurrence was the most strongly associated factor in the choice of mastectomy over BCS.

P1-11-04
Breast Cancer Treatment Resources and Guideline-Concordant Treatment in Appalachia.
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Background: Appalachia has poorer cancer outcomes, but little research has been done regarding availability of cancer care resources in this region and how resource availability may relate to cancer outcomes. This study 1) examines associations between radiation therapy resources and receipt of radiotherapy after BCS in counties within Kentucky - a SEER state; and 2) describes spatial patterning of breast cancer treatment resources in all 13 Appalachian states. Methods: For the Kentucky analyses, county-level data from the Area Resource File and SEER registry are analyzed. Bivariate analyses and spatial lag regression using a 6-nearest neighboring counties matrix are conducted. The sample includes stage I or II primary breast cancer patients age 18+ years diagnosed in Kentucky during 2000-2007. The dependent variable is the county-level percentage of patients received BCS without radiation; independent variables include density of radiation therapy providers and facilities and other socioeconomic covariates. For the analyses of entire Appalachian region, descriptive analyses and exploratory spatial data analysis are conducted including 420 Appalachian counties and 644 non-Appalachian counties in 13 states. Results: In Kentucky 16.44% of 17,227 early stage breast cancer patients received BCS without radiation therapy (21.08% in Appalachian versus 14.80% in non-Appalachia, p<0.001). Appalachian Kentucky had significantly fewer radiation oncologists and radiation therapy facilities per capita than non-Appalachian Kentucky. The number of radiation therapy facilities per capita is negatively associated with rates of BCS without radiation when controlling for covariates. Analysis of 13 Appalachian states shows that Appalachian counties, especially in the Central and Southern regions, had significant fewer physicians per capita in Surgery, Anesthesia, Clinical Pathology, and Radiation Oncology. Clustering of scarce breast cancer care resources was observed in Central Appalachia. Conclusions: Appalachian counties, especially in central Appalachia, have fewer breast cancer treatment resources than non-Appalachian counties, and resource availability is associated with cancer health disparities.
P1-11-05

Investigation of Epidemiological Factors as Barriers to Indicated Radiation Therapy in Post-Mastectomy Breast Cancer Patients in South Carolina.

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Purpose: Post-mastectomy radiation therapy (PMRT) has been shown to provide improved locoregional control and overall survival in intermediate and high-risk breast cancer patients. To investigate the frequency of utilization and potential barriers to receipt of adjuvant PMRT in South Carolina, an analysis of the S.C. Cancer Registry (SCCR) database was undertaken.

Methods: Epidemiological factors such as age, race, and geographic distance from a radiation therapy facility were analyzed as potential predictors of whether patients received the indicated radiotherapy. Post-mastectomy breast cancer patients (n=1080) recorded in the SCCR from 2006-2008 were stratified as low (Tis/T1, N0) (43%), intermediate (T1/T2, N1) (27%), or high (T3/T4, N2/N3) (30%) risk. Logistic regression models were used to predict receipt of adjuvant radiotherapy based on factors such as race, age, and geographical distance from radiation treatment facility.

Results: Adjuvant PMRT was received in 4% (19/469) of low risk patients, 23% (67/288) of intermediate risk patients, and 44% (141/323) of high risk patients. In both intermediate and high risk groups, older patients were less likely to receive PMRT. The odds of receiving PMRT decreased by 3% (OR=1.03; 95% CI: 1.01-1.05; p=0.01) and 2% (OR=1.02; 95% CI: 1.00-1.03; p=0.03) per year in the intermediate and high risk groups, respectively. Race and distance from treatment facility were not predictive of receipt of PMRT in high risk patients.

Conclusion: Utilization of adjuvant PMRT in intermediate and high risk breast cancer patients in South Carolina is low, despite the evidence that it confers a survival benefit in both premenopausal and postmenopausal women. Of the patient-related factors analyzed, only age predicted lower odds of receiving radiotherapy in both the intermediate and high risk group. In high risk patients of advanced age (>70), breast cancer continues to be a major cause of mortality which can be improved by radiotherapy. Future educational efforts should focus on addressing patient and physician bias against radiotherapy based on patient age. Further study is needed to identify socio-economic-, physician- and health system-related factors that may be impacting the underutilization of PMRT in South Carolina.

References:

P1-11-06

Barriers to Enrollment in Cancer Therapeutic Clinical Trials: A Comprehensive Cancer Center Experience.

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PURPOSE: Well-conducted cancer therapeutic clinical trials are essential for improving patient outcomes. Unfortunately, less than 5% of new cancer patients participate in clinical trials on average nationwide. Failure to recruit eligible patients represents a major impediment to the success of clinical trials. Barriers to patient accrual in cancer clinical trials must be identified and overcome to increase patient participation.

MATERIALS AND METHODS: A retrospective analysis of 418 patients with a diagnosis of breast cancer seen at City of Hope Medical Center in 2009 was performed. Along with descriptive analysis of patient demographic data, logistic regression analyses were performed to evaluate predictors of enrollment.

RESULTS: Of the 418 new breast cancer patients in 2009, we determined that 138 (33%) were eligible for available therapeutic clinical trials at City of Hope. At the initial visits, physicians did not consider clinical trials in 32% (44/138) of these patients. Of the 138 eligible patients, 58% (80/138) of patients participated in clinical trials. The remainder (58/138, 42%) either declined trial participation despite meeting eligibility criteria (14/58, 24%), or were not considered for clinical trials by their respective physician (44/58, 76%). By logistic regression analysis, patients with Stages II and III disease were significantly more likely to enroll in clinical trials compared to those with stage 0 or I (OR=2.89, 95% CI, 1.17-7.12, P = .02; OR=9.17, 95% CI, 2.65-31.76, P = .0005; respectively). Age, preferred language, marital status, family history of breast cancer, and race were not found to be significant predictors of enrollment in therapeutic clinical trials.

CONCLUSION: While enrollment of eligible breast cancer patients onto therapeutic clinical trials at City of Hope is high (58%), overall the proportion going onto clinical trials remains low (80/418, 19%), though higher than national averages. We found that the majority of patients did not enroll due to lack of availability of a suitable trial, ineligibility, or lack of offering of trial enrollment by the treating MD. Barriers to cancer clinical trial accrual should be prospectively identified and addressed in future studies, with the hope of leading to more vigorous enrollment.
Impact of Reduction in Cost-Sharing on Screening Mammography Utilization among Rural U.S. Women.


Background: Early detection of breast cancer through mammography screening leads to earlier stage at diagnosis and improved survival. For reasons that are poorly understood, in recent years the rate of screening has demonstrated periods of decline, and screening has proved to be less common in rural compared to urban areas of the U.S. In 2006, the National Rural Electric Cooperative Association (NRECA) which provides health care to over 100,000 electrical workers and their families in primarily rural areas of the U.S. eliminated copayments for screening mammography in an effort to boost screening rates.

We conducted a population based analysis of screening utilization to determine the impact of this policy initiative.

Methods: Using the NRECA insurance database, all women aged 40 to 64 with no prior history of breast cancer or DCIS (based on ICD-9 codes) were identified and we evaluated claims data on annual screening mammography utilization (SMU) between 1999 and 2009 stratified by age group in 5 year intervals. Changes in SMU over time were assessed focusing on the periods before and after the policy change in January 2006. We also evaluated diagnosis of breast cancer and receipt of mastectomy and chemotherapy as a potential proxy for more advanced disease at diagnosis. Descriptive statistics were estimated and the mammography rate was fitted on years using the identity link (proc genmod in SAS). In order to test the impact of the 2006 change in cost-sharing on the trend in mammography rate, we introduced change point terms in slope and intercept to the linear model. Chi-squared test for 2x2 tables was used to compare SMU rates between two consecutive years for each age group. All p-values are two-sided.

Results: During this period, a mean of 20,825 women aged 40 to 64 each year received health insurance through NRECA. SMU increased from 38.1% in 1999 to 49.5% in 2009. Analyzing SMU before and after the change in cost sharing policy demonstrates a significant change in the rate of screening at the 2006 intercept (p = 0.0275) although the slope of year to year change in screening rate did not change. In stratified analysis there was a significant change in SMU between 2005 and 2006 for all age groups. In the 4 years prior to the NRECA change in policy, 554 women were diagnosed with breast cancer and 40 underwent mastectomy and chemotherapy. In comparison, from 2006 to 2009, only 20 women out of 540 with newly diagnosed breast cancer underwent such therapy (7.2% prior to policy change vs. 3.7% following, P = 0.01).

Conclusion: The impact of health plan benefits changes can be evaluated among a primarily rural population of women aged 40 to 64 using the novel NRECA database. Annual SMU remained low, but improved following elimination in copayments suggesting that financial barriers impact screening. Multiple factors may explain changes in SMU and treatment intensity over time. However, cost-sharing for high value health care services may have unintended negative consequences. Further evaluation of this database is planned to evaluate biannual screening rates, correlation with sociodemographic factors, impact of recent controversy over screening guidelines and additional barriers to screening utilization in this rural population.

Breast Cancer Screening Resources and Stage at Diagnosis in Appalachia: A Geospatial Perspective.

Yao N, Hillemeier MM, Anderson RT. Penn State, University Park, PA; Penn State, Hershey, PA

Background: National Cancer Institute has designated Appalachia as a priority area characterized by significant disparities in cancer outcomes. However, little research focuses on the availability of cancer care resources in this region and how resource availability may relate to cancer outcomes. This paper will describe the distribution of breast cancer screening resources in Appalachia and examine the relationship between the screening resources and breast cancer stage at diagnosis at the population level.

Method: Percentages of early stage breast cancer incidence are computed based on the county-level data from the four Appalachian state cancer registry data from 2000 to 2008. Per capita breast cancer screening providers or facilities are computed for these Appalachian states. Descriptive analysis, exploratory spatial data analysis, and spatial regression were conducted.

Results: Appalachian counties had significantly fewer primary physicians, OB/GYN specialists, and diagnostic radiologists per capita than non-Appalachian counties. Spatial analysis demonstrates moderate clustering of scarce breast cancer screening resource and low percentage of early stage breast cancer incidence in West Virginia and Appalachian Kentucky. Those diagnosed at early stages were 67.92% of Appalachian vs. 68.34% of non-Appalachian breast cancer patients. The number of diagnostic radiologists per capita is significantly associated with the percentage of early stage breast cancer incidence when controlling for covariates such as county level poverty rates, uninsurance rate, percentage of adults with college degree, and per capita primary care resources.

Conclusions: Fewer diagnostic radiologists in West Virginia and Appalachia Kentucky are associated with lower percentage of early stage breast cancer incidence in the Central Appalachia region. Public health intervention could include policies and regulations to improve breast cancer screening resources in this region.

Table 1. Regression Analysis Modeling Variables Predicting for Percentage of Early State Breast Cancer Incidence at County Level

<table>
<thead>
<tr>
<th>Variables</th>
<th>Coefficient (Std. Error)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>-0.02 (0.01)</td>
</tr>
<tr>
<td>Appalachian designation</td>
<td>-0.02 (0.01)</td>
</tr>
<tr>
<td>Number of primary physicians per 100,000 residents</td>
<td>-0.02 (0.01)</td>
</tr>
<tr>
<td>Number of OB/GYN per 100,000 residents</td>
<td>-0.02 (0.01)</td>
</tr>
<tr>
<td>Number of diagnostic radiologists per 100,000 residents</td>
<td>-0.02 (0.01)</td>
</tr>
<tr>
<td>Number of hospitals with mammography service per 100,000 residents</td>
<td>-0.02 (0.01)</td>
</tr>
<tr>
<td>Percentage of white population</td>
<td>-0.02 (0.01)</td>
</tr>
<tr>
<td>Poverty rates</td>
<td>-0.02 (0.01)</td>
</tr>
<tr>
<td>Percentage of uninsured population</td>
<td>-0.02 (0.01)</td>
</tr>
<tr>
<td>Percentage of adults with college degree</td>
<td>-0.02 (0.01)</td>
</tr>
</tbody>
</table>

Notes: * p<.05

Effects of a Multidisciplinary Breast Cancer Clinic in an Appalachian Based Medical Center.

Ingham JA, Creameans DK, Daugherty SL, Myhand RC, Sever WE. Adena Health System, Comprehensive Breast Care Program, Chillicothe, OH

Background: Adena Health System is a not for profit 250 bed institution that provides health care for a 13 county region in southeast, Appalachia Ohio. Access to health care in rural setting is an ongoing challenge. Several barriers to health care access affect this region. Socioeconomic burden and lack of higher education are two of the most dominant barriers. The age-adjusted breast cancer rates are 5.3% higher in the Appalachian region and the stage at presentation is more...
advanced. In December of 2009, we held our first Multidisciplinary Breast Cancer Clinic (MDBCC). The goal of the MDBCC is to facilitate prompt diagnosis and effective treatment. The MDBCC is staffed by a team of physicians consisting of surgeons, medical oncologists, and radiation oncologists. The team meets with the patient in a one on one setting. That same day the patients are discussed by the team with input from pathology and radiology in order to tailor a treatment plan specifically for each patient. After the conference, the recommendations are discussed with the patient before treatment is initiated.

Material and Methods: In a retrospective review, we evaluated the effect of a MDBCC on days to diagnosis, days from diagnosis to treatment, percentage of patients receiving neoadjuvant chemotherapy, and stage at diagnosis. Since December 2009, there have been over 90 breast cancer patients evaluated in the MDBCC. We randomly selected 60 breast cancer patients treated prior to MDBCC between 2008-09 and 66 patients evaluated at the MDBCC between December 2009-2011 from the tumor registry database.

Results: The average number of days to diagnosis pre-MDBCC was 15.5 days as compared to 4 days post-MDBCC. In 2008-09, the average days to treatment (pathology result to a-port placement, surgery or chemotherapy) was 18 days as compared to 12 days post-MDBCC. The percentage of patients receiving neoadjuvant chemotherapy has not changed significantly, however, there is an upward trend approaching statistical significance (10% per-MDBCC vs. 12% post-MDBCC). The stage at presentation has not changed significantly since the start of the MDBCC.

Discussion: The development of the MDBCC has proven to be a successful team oriented approach to breast cancer diagnosis and treatment. Time to diagnosis and initiation of treatment has improved. Providing patients with access to multiple specialists and forming a treatment plan the same day improves quality of care and lessens economic burden. The MDBCC is clearly feasible and particularly suited to the rural hospital setting. System wide, we hope to see a decrease in the stage at presentation and are confident based upon our current data that this is a realistic goal.

P1-11-10
Quality of Breast Cancer Care in a Boston Area Patient Navigator Program.
Raj A, Ko N, Battaglia T, Moy B. Massachusetts General Hospital, Boston, MA; Boston Medical Center, Boston, MA

Background: Elimination of disparities is critically important for lessening the burden of cancer. Patient navigator programs (PNPs) assist with all aspects of care, including access, cancer prevention, screening, post-diagnosis care, and survivorship care. Little is known about the effect of PNPs on patient care and outcomes following the diagnosis of breast cancer (BC). We examined quality measures (QMs) of breast cancer care among women participating in the Massachusetts General Hospital Avon Breast Care Patient Navigator Program (MABCP), which provides patient navigation services to disadvantaged minority communities in the greater Boston area.

Methods: Women diagnosed with BC who participated in the MABCP from 2001 to 2011 were followed to determine the proportion whose care was concordant with American Society of Clinical Oncology/National Comprehensive Cancer Network (ASCO/NCCN) QMs. QMs included 1) hormonal therapy (HT) within 1 year of diagnosis for HR+ tumors > 1 cm; 2) chemotherapy within 120 days of diagnosis of HR- >1cm tumors for women <70 years; and 3) post-lumpectomy radiation therapy (XRT). Descriptive statistics were used to report characteristics of MABCP patients.

Results: Of the 186 MABCP patients diagnosed with BC, some treatment data was available on 158 (85%) and race/stage information was available on 149 (80%) [Table 1]. Among the MABCP patients, concordant care was received by 70/74 (95%) for the HT QM, 15/17 (88%) for the chemotherapy QM, and 65/71 (92%) for the XRT QM. In comparison, available benchmark concordance rates of BC patients treated at 8 NCCN centers from 2003-6 are: 340/382 (89%) for the HT QM, 156/179 (87%) for the chemotherapy QM, and 141/148 (95%) for the XRT QM.

Conclusions: Overall, breast cancer care in the MABCP PNP is concordant with published ASCO/NCCN quality measures. At present, the sample is insufficient to compare concordance rates with NCCN patients but preliminarily, it appears that the quality of care is comparable. Future research should include prospective analyses of quality metrics to assess the process and outcomes of patient navigation in diverse settings, compared with control populations.

P1-11-11
Wait Times for Breast Cancer Care in Manitoba 2009-2010. Time To Face the Challenge.
Carpenter-Kellett T, Nashed M. CancerCare Manitoba, Winnipeg, MB, Canada

Background: Wait times for patients with breast cancer to receive oncological treatment vary significantly. In Manitoba, the radiation treatment wait times improved in the year 2005 compared to 2001. However, other parts of the patient’s journey have lengthened and negated the reduction seen in radiation treatment wait times.

Aims: To examine the overall time from disease suspicion to treatment of breast cancer patients from June 2009 to June 2010 and to compare to the previously published wait times.

Methods: This population-based retrospective study looked at representative samples of women newly diagnosed with breast cancer. Patients were followed from the time of first presentation, either to their family physician or after a suspicious screening mammogram, to the time adjuvant treatment was started. Each patient’s journey was subdivided into different chronological stages. The data was compared to the wait times reported in 2005.

Results: 363 patients’ data was collected and analyzed. The median elapsed time in days for each phase of the journey was calculated. Total wait times from suspicion to diagnosis were also calculated. The wait times of most stages of the patients’ journey have worsened when compared to 2005. However, there was some improvement in the diagnostic part of the journey. Delays to surgery and pathology have contributed to lengthening of the total journey. There was also a systematic and repetitive administration delay at each transition. Screen-detected cases had a shorter journey than those who presented through a family physician.

Conclusions: In spite of improvements achieved in the wait times at some stages of the journey, the total length of the patient journey has not been shortened. This represents not only individual team failures but also a built in complexity in the health system. In order to achieve a meaningful cut in wait times for breast cancer patients, the entire trajectory of care needs to be addressed.
**P1-11-12**

Patterns of Care of Newly Diagnosed Patients with Breast Cancer in Mexico.

Chavarrí-Guerra Y, Liedke PER, Symecko H, Hammond EE, Higgins MJ, Finkelstein D, Goss PE. Massachusetts General Hospital, Boston, MA

Background: Breast cancer is the most common form of cancer and the leading cause of cancer death in women worldwide. More than 55% of breast cancer deaths occur in low and middle income countries. Although incidence rates for breast cancer are lower in developing countries, mortality rates are higher. This phenomenon has been attributed to limited access to care for breast cancer patients, including screening and early diagnosis as well as primary surgical, radiation and systemic therapies. Similar to trends in other poor and middle income countries, breast cancer mortality in Mexico is rising. The goal of this survey of physicians caring for patients with breast cancer in Mexico is to obtain information about current treatment patterns of newly diagnosed patients and to describe their clinical characteristics.

Methods: A web-based closed survey has been sent to 854 physicians providing care to newly diagnosed breast cancer patients across Mexico, including medical oncologists and breast cancer surgeons. The survey instrument contains 35 questions assessing demographic data, access to diagnosis and treatment in a variety of clinical patient scenarios. The responses will be anonymous and entered automatically into a secure database for analysis. Fisher exact test will be used for the frequency analysis. Chi-squared statistics and Kendall correlation will be used for nominal and ordinal variables respectively.

Results: The results will be presented at the 2011 San Antonio Breast Cancer Symposium.

Conclusions: The results of this survey will highlight potential disparities in care received by breast cancer patients across the full geographic and socioeconomic spectrum of Mexico in order to highlight the need for uniform, quality based approaches for the diagnosis and treatment of breast cancer patients in Mexico, and will serve as an example of how one middle income country faces challenges and unmet medical needs regarding access to care of women with breast cancer.

**P1-11-13**

Improvement in the Quality of Care for Patients with Locally Advanced Breast Cancer through Implementation of an Integrated Electronic Care Pathway.

Hogeveen S, Han D, George RL, Sweet-Goldstein M, Dinnwell RE, Muradali D, Brezden CB, Haq R, Simmons CE. St. Michael’s Hospital; Princess Margaret Hospital

Background: Locally advanced breast cancer (LABC) refers to the most advanced stage of non-metastatic tumours with an incidence of approximately 10% in newly diagnosed breast cancers. Currently, for optimal care, patients with LABC require a multidisciplinary approach including coordinated planning with medical, surgical and radiation oncologists. Many barriers may exist in co-ordinating timely management of these patients. Care pathways are important tools to streamline care and data collection and feedback allows for quality improvement in clinical practice. We created an interactive electronic care pathway and self-populating quality assurance database at St. Michael’s Hospital (SMH) to facilitate multidisciplinary teams to track LABC patient histories and patient treatments in order to coordinate therapy effectively and expedite care (LABC E-PATH).

Methods: This is an observational before-and-after cohort study of patients with LABC with a retrospective review pre-implementation and prospective collection of clinical data post-implementation. The completeness of workup and the timeliness of treatment pre- and post-implementation were assessed.

Results: With the implementation of the LABC E-PATH in May 2010, the time between the referral of an LABC patient to a medical oncologist and consultation has decreased from 7 days (range 0 to 493) pre-implementation to 2 days (range 0 to 29) post-implementation. The time between referral to medical oncologist and the start of their chemotherapy treatment decreased significantly from a median of 19 days to 8 days (pre-implementation: range = 0 to 493, post-implementation: range = 0 to 128) (p=0.0028). All pre-treatment staging was completed faster post-implementation of the LABC e-path than pre-implementation, expediting time to initiation of chemotherapy. The number of referrals for LABC to the SMH program increased from < 1 patient per month to 4 patients per month post-implementation.

Conclusions: The LABC E-PATH has achieved its goal of expediting care and overcoming barriers to timely treatment in this patient population. It has also ensured timely and appropriate resource allocation. This unique system may also be applied to other disease sites where coordination of a multi-disciplinary team is critical for appropriate patient management.

**P1-11-14**

Gender and Sexual Orientation of Clients Who Were Linked to Breast Cancer Screening Services through Outreach and Education Supported by the Avon Breast Health Outreach Program.


Background: Lesbian, gay, bisexual, and transgender (LGBT) individuals experience health disparities in breast cancer screening related to multiple socioeconomic factors, including stigma and discrimination. Since 2001, the Avon Breast Health Outreach Program (Avon BHOP) has granted almost $60 million to 240+ organizations to promote breast cancer screening. Avon BHOP beneficiaries collect a standardized set of de-identified, self-reported client level health and demographic data to ensure that programs reach their target population. Prior to 2011, no data on client gender or sexual orientation were collected.

Methods: From 2009-2010, the Avon BHOP conducted a review of the standard confidential client intake form (CIF), last revised in 2006. Based on stakeholder input, including the Avon Foundation for Women and Avon BHOP beneficiaries, the CIF was updated in 2011 to incorporate variables for gender and sexual orientation. Proposed revisions were presented to current beneficiaries in June 2010 for feedback. Although the CIF has historically included many sensitive elements such as race/ethnicity and income, several programs expressed concern that clients or program staff would be uncomfortable addressing questions on gender and sexual orientation in particular. In response, the Avon BHOP Coordinating Center provided standard language that programs could use to help ease the concerns of staff and clients that incorporated three key messages: 1) the same form is used for all clients and programs funded by the Avon BHOP to help ensure funds are reaching clients in need; 2) all responses are kept confidential; and 3) clients may skip any question they are not comfortable answering.

Results: Preliminary CIF data for January-March 2011 were analyzed for 20,672 clients from 109 programs. Overall, 96.6% (n=19,966) indicated female gender; 0.5% (n=101) self-reported gender as
follows: 75 male, 8 transgender, 12 “other”, and 6 “unknown”; and 2.9% (n=605) did not respond to this question. For sexual orientation, 77.7% (n=16,067) of clients selected “heterosexual”. Of the remaining 22.3% (n=4,606), 175 (0.8%) selected “lesbian, gay or bisexual”, 431 (2.1%) selected “other”, 1045 (5.1%) selected “unknown”, and 2,954 (14.3%) did not respond to this question. By program, the non-response rate ranged from 0% to 40.2% for gender, and from 0% to 81.9% for sexual orientation.

Discussion: Avon BHOP programs were able to collect sensitive information on client gender and sexual orientation during the first three months during which this information was requested. Despite concerns expressed among staff, overall response rates were high and rates of “unknown” (which may indicate that a staff person did not ask a client to complete the question) were low. Beneficiaries receive a quarterly summary of their own CIF data, which allows program staff to review client responses and ensure that client needs are met in a culturally sensitive manner. The Avon BHOP is committed to supporting diversity among funded programs; requesting data on client gender and sexual orientation may help improve services for LGBT clients by giving them a voice and reducing stigma.

P1-12-01
Pregnancy during and Following Adjuvant Trastuzumab in Patients with HER2-Positive Breast Cancer: An Analysis from the HERA Trial (BIG 01-01).
Azim, Jr HA, Metzger-Filho O, de Azambuja E, Loibl S, Focant F, Gresko E, Procter M, Piccart-Gebhart M. Institut Jules Bordet, Brussels, Belgium; German Breast Group, Frankfurt, Germany; F. Hoffmann-La Roche, Basel, Switzerland; Frontier Science, Kincraig, United Kingdom

Background: 1-year of adjuvant trastuzumab (T) is the standard of care in managing patients (pts) with early HER2-positive breast cancer (BC). As T is not known to alter fertility, pts with childbearing potential could become pregnant during or following treatment with T. Cases of oligohydramnios, some associated with fatal pulmonary hypoplasia of the fetus have been reported in women receiving T during pregnancy (preg). Here we report the outcome of preg in all pts enrolled in the HERA trial.

Methods: Pregnancies in the HERA trial are reported on a distinct “pregnancy form” for up to 10 years following T completion. The form includes information on approximate date of conception, preg course & outcome, fetal measurements at birth, and congenital anomalies. Any missing data were retrieved from the study site, if available. For this analysis, pts were grouped into 3 groups: 1) preg during and up to 3 months after T, 2) preg > 3 months of last T dose, and 3) preg with no prior exposure to T.

Results: By March 2010, 70 preg were reported in 58 out of 5102 pts randomized. Five, 30 and 7 completed preg were reported in groups 1, 2 & 3 respectively.

Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>(Preg on T)</th>
<th>(Preg after T)</th>
<th>(Preg with no exposure to T)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pts (n.preg)</td>
<td>16 (16)</td>
<td>31 (43)</td>
<td>11 (11)</td>
</tr>
<tr>
<td>Missing preg info</td>
<td>0</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>N.completed preg</td>
<td>5 (31%)</td>
<td>30 (98%)</td>
<td>7 (63%)</td>
</tr>
<tr>
<td>Live Birth</td>
<td>5 (100%)</td>
<td>33 (100%)</td>
<td>7 (100%)</td>
</tr>
<tr>
<td>Mean gestational week at delivery</td>
<td>40 (30)</td>
<td>38 (22)</td>
<td>39 (6)</td>
</tr>
<tr>
<td>Mean Apgar score at 10 min</td>
<td>10 (3)</td>
<td>9.5 (18)</td>
<td>9 (9)</td>
</tr>
<tr>
<td>Mean fetal weight in gm</td>
<td>3485 (34)</td>
<td>3125 (25)</td>
<td>3431 (7)</td>
</tr>
<tr>
<td>Mean fetal length in cm</td>
<td>50 (3)</td>
<td>52 (18)</td>
<td>50 (9)</td>
</tr>
</tbody>
</table>

* 3 pts got preg on and after T ** 3 twin deliveries

As per protocol, all pts on T were required to use adequate contraceptive measures, yet 16 pts became pregnant during the course of T and up to 3 months thereafter. The percentage of completed preg was lowest in group 1, with 4 spontaneous and 7 induced abortions. In group 2, preg occurred at a mean of 29 months following completion of T, with 6 spontaneous and 4 induced abortions. In group 3, abortion was induced in 3 pts and no spontaneous abortions reported. Across all 3 groups, all but 1 spontaneous abortion occurred during the 1st trimester. Two congenital anomalies were reported; a Down’s syndrome in a 43 year old pt >5 years after completing T for which abortion was induced, and one with partial fusion of the 2nd and 3rd toe born to a pt in group 3.

Conclusions: Unintentional exposure to T during preg may be associated with spontaneous abortion, yet the numbers remain low to draw firm conclusions (spontaneous abortion rate in general population is up to 20%). No oligohydramnios or anomalies were observed in group 1. While an increased risk of oligohydramnios has been reported when T is administered after the 1st trimester, T administered to Cynomolgus monkeys during organogenesis did not cause fetal harm (Pentsuk et al; 2009). Nevertheless, women of childbearing potential should be advised to use effective contraception during and up to 6 months after treatment with T. On the other hand, prior exposure to T did not appear to affect the preg course or outcome. We are planning to collect information from the other T adjuvant trials to confirm our findings.

P1-12-02
Patient-Reported Outcomes (PROs) from a Randomized Phase II Study (TDM4450g/B021976) of Trastuzumab Emtansine (T-DM1) vs Trastuzumab Plus Docetaxel (HT) in Previously Untreated HER2-Positive Metastatic Breast Cancer (MBC).
Bianchi GV, Koecis J, Dirix L, Toriboe Y, Lalla D, Tong YB, Guardino AE, Hurvitz SA. Fondazione IRCCS Istituto Nazionale Tumori, Milan, Italy; Semmelweis University Budapest, Budapest; Sint-Augustinus, Antwerp; Genentech; UCLA/Translational Oncology Research International

Background
T-DM1 is an antibody-drug conjugate in development for HER2-positive cancer. As a single-agent, it has demonstrated promising efficacy and safety when administered after multiple lines of HER2-directed therapy. In the current study, we compared the safety and efficacy of T-DM1 to standard first-line treatment for HER2-positive MBC. This abstract highlights the PRO results in the context of efficacy and safety.

Methods
Patients with HER2-positive MBC and no prior MBC therapy were randomized 1:1 to T-DM1 3.6 mg/kg IV q3w or HT 6 mg/kg IV (8 mg/kg in cycle 1) + T 75 or 100 mg/m2 IV q3w. Primary objectives were investigator-assessed progression free survival (PFS) and safety. Key secondary end points included objective response rate and the FACT-B Trial Outcome Index (TOI). Patients completed the FACT-B on day 1 of each treatment cycle. The FACT-B TOI, the primary PRO end point, comprises a subset of the FACT-B and provides a summary measure of physical and functional well being and breast cancer specific symptoms. Time to FACT-B TOI worsening (ie, ≥5 point decrease in TOI) was assessed with Kaplan-Meier methods and a Cox model. A repeated measures mixed effects (RMME) model was used to evaluate potential treatment effects on TOI across cycles. Preliminary PRO results based on a data cutoff of November 15, 2010, are included here; final data will be presented.

Results
A total of 137 patients were randomized (67 T-DM1, 70 HT). Baseline patient and disease characteristics were similar in each arm. Patients treated with T-DM1 had longer PFS (14.2 vs 9.2 months, HR=0.59, P<0.035) with less toxicity (eg, 46.4% grade 3/4 adverse events
with T-DM1 vs 89.4% with HT) than those treated with HT. ORR was similar in both arms (64.2% T-DM1, 58.0% HT). A total of 132 patients (65 T-DM1, 67 HT) were evaluable for PRO analyses with a high compliance rate. In a preliminary analysis, FACT-B TOI worsening was significantly delayed in the T-DM1 arm compared with the control arm (7.5 vs 3.5 months, HR=0.58, \(P=0.022\)). The RMME model showed a mean difference of 3.65 in FACT-B TOI scores (\(P=0.023\)), mainly driven by physical well being (PWB) scores (mean difference 2.28, \(P=0.002\)), favoring T-DM1. In addition, 5 of 7 PWB items showed significantly better mean scores in the T-DM1 arm: “lack of energy” \((P=0.011)\), “trouble meeting needs of family” \((P=0.025)\), “bothered by side effects” \((P<0.001)\), “feeling ill” \((P=0.016)\) and “forced to spend time in bed” \((P=0.015)\); the 2 remaining items, “nausea” and “pain,” showed numerically better mean scores with T-DM1.

Conclusions

Compared to HT, T-DM1 as first-line treatment of HER2-positive MBC conferred longer PFS and a more favorable toxicity profile. The PRO data suggest that T-DM1 is also associated with meaningfully improved tolerance relative to HT, contributing to an overall clinical benefit and better health-related quality of life. Thus, T-DM1 may improve the standard of care for patients with previously untreated HER2-positive MBC.

P1-12-03

Combined Targeting of the PI3K Pathway and HER2 Overcomes Acquired and De Novo Trastuzumab Resistance.

O’Brien NA, McDonald K, Von Eiw E, Conklin D, Kalous O, Di Tomaso E, Finn RS, Slamon DJ, University of California at Los Angeles, Los Angeles, CA; Novartis-Oncology, Cambridge, MA

Background: Although trastuzumab and lapatinib provide clinical benefit for women with HER2-positive breast cancer, both de novo and acquired resistance to these agents exist. There is increasing evidence to suggest that aberrant activity of the PI3K/akt/mTOR signaling pathway is one of the key mechanisms responsible for resistance. Thus, pharmacologically targeting the PI3K pathway is a rational approach for overcoming resistance to HER2-targeted therapy. However, specifically targeting individual elements of the PI3K pathway may result in feedback activation or in activation of compensatory pathways, whereas targeting the pathway at multiple points may be more effective in permanently shutting down proliferation and inducing cell death.

Materials and Methods: In this study we evaluated the activity of three small molecule tyrosine kinase inhibitors that target specific critical portions of the PI3K pathway; BKM120 (pan-PI3K), RAD001 (mTORC1 specific) and BEZ235 (dual PI3K & mTOR) both individually and in combination with trastuzumab in a panel of HER2-amplified breast cancer cell lines that have previously been characterized for trastuzumab response/resistance.

Results: In the trastuzumab sensitive BT-474 and SKBR3 cell lines, 72 hours of trastuzumab treatment efficiently inhibited cell proliferation and deactivated AKT (S473 & T308), pS6K and ERK signaling. In contrast, the trastuzumab-resistant MDA453 and SUM225 cell lines, as well as the BT-474 cells conditioned to acquire trastuzumab resistance (BT-TR), trastuzumab treatment did not inhibit pAKT, pS6K and pERK levels. RAD001 had significant antiproliferative activity in both trastuzumab sensitive and resistant cell lines and resulted in rapid down regulation of pS6K (mTOR) activity (15 min). However, this deactivation was followed by a reactivation of AKT and ERK signaling (24 to 72 hours). BKM120 also demonstrated anti-proliferative activity independent of trastuzumab sensitivity. Specifically targeting PI3K with BKM120 directly decreased pAKT/pS6K/pERK signaling and feedback activation was less pronounced than that observed with RAD001. Of the three molecules tested, BEZ235 was the most efficacious in the cell line panel (all IC50gs < 20 nM). BEZ235 rapidly (15 min) decreased pAKT/pS6K/pERK signaling in both trastuzumab sensitive and resistant cell lines and this inhibition was maintained at 72 hours. Thus, BEZ235 is effective in shutting down PI3K feedback activation and efficiently shuts down PI3K signaling in trastuzumab resistant HER2-amplified breast cancer cell lines. The combined treatment of trastuzumab and each of the PI3K/mTOR targeting agents resulted in greater inhibition of proliferation and induction of cell death in the HER2-amplified cell lines than each agent used alone. No increase in activity was observed in the HER2-normal control cell lines, MCF-7 and KPL-1.

Discussion: Together, these data indicate that targeting the PI3K/akt/mTOR pathway either alone or in combination with trastuzumab is effective in overcoming trastuzumab resistance. These findings are currently being validated in in vivo models of trastuzumab resistance.
in a dose-dependent manner respective to parental cells. Interestingly, shEBP50 cells demonstrated an enhanced capacity to form mammospheres compared to vector control cells. We demonstrate that EBP50 is able to bind HER2 using immunoprecipitation, suggesting that EBP50 interacts directly with HER2. Confocal microscope analysis demonstrated the colocalization of these two proteins. Inhibitors to c-Src, PI3K, AKT and EGFR were used in combination with Tras; shEBP50 cells were sensitive to both PI3K and AKT inhibitors, and EGFR inhibitors were able to restore Tras sensitivity. 

Discussion: Our data suggest that EBP50 is a novel negative regulator of HER2 signaling, and its loss conferred resistance to both Tam and Tras. EBP50 loss might function to stabilize HER2, and enhance dimerization with EGFR and HER3. We hypothesize that EBP50 levels might be a new predictive biomarker for targeted therapy; patients with low EBP50 levels might best be treated with a combination of therapies including PI3K/AKT inhibitors.

P1-12-05
Complete Pathological Response of Ductal Carcinoma In Situ after Treatment with Neoadjuvant Herceptin.
Chalmers CR, Mallon EA, Tougan N, Horgan KJ, MacPherson I, Doughty JC. The Western Infirmary, Glasgow, Scotland, United Kingdom; Leeds General Infirmary, Leeds, Yorkshire, United Kingdom

Introduction: The introduction of neo-adjuvant herceptin has led to pathological complete response rates of up to 60% in patients with breast cancer. The traditional (NSABP) definition of pathological complete response includes cases demonstrating residual DCIS and little is known about the effects of neo-adjuvant herceptin in this situation. Current evidence suggests that DCIS is chemo-resistant, but it has also been shown that DCIS is more likely to be HER-2 positive than primary cancer.

Results: We describe six patients who received neo-adjuvant chemotherapy and herceptin for biopsy-proven, node-positive, large breast cancers either with an extensive component of concomitant DCIS or a separate DCIS focus. Neo-adjuvant treatment did not result in any changes in the pattern of mammographic calcification seen pre-operatively (Figure 1 a-c), however histopathology of all six specimens showed ductal spaces containing macrophages and calcification but no residual lining epithelium, features consistent with pathological complete response of the DCIS component (Figure 1 d).

Figure 1: Pathologically complete response of DCIS after neo-adjuvant treatment with herceptin. (a) Pre-treatment mammogram (b) Post neo-adjuvant chemotherapy and herceptin mammogram showing resolution of the mass effect of the primary cancer and unchanged residual mammographic calcification associated with the extensive DCIS component (c) Wire-localised specimen demonstrating excision of calcification (d) Histopathology of the same specimen showing calcium-filled ducts and no residual lining epithelium.

Discussion: In conclusion, neo-adjuvant herceptin is associated with complete pathological response of both breast cancer and DCIS. The presence of unchanged persistent mammographic calcification in known regions of DCIS after treatment complicates any role of mammographic surveillance in this group and raises the question of optimal surgical management in the future management of these individuals.

P1-12-06
The Role of MAPK and PI3K/AKT/mTOR Signaling in Innate Lapatinib Resistance.
McDermott M, O’Brien N, McDonald K, Crown J, O’Donovan N, Slamon D. Dublin City University, Glasnevin, Dublin 9, Ireland; University of California Los Angeles, Los Angeles, CA; St. Vincents University Hospital, Elm Park, Dublin 4, Ireland

Background: Lapatinib is a tyrosine kinase inhibitor which blocks downstream MAPK and PI3K/AKT/mTOR proliferation and survival signaling pathways in HER2 positive breast cancer cell lines, tumor xenografts and HER2 positive breast cancer patients. However, pre-clinical and clinical studies have shown that not all HER2 positive patients respond to lapatinib and thus innate resistance to lapatinib exists. The identification of biomarkers of lapatinib response is therefore critical and would enable individual therapeutic decisions to be based on tumor biology rather than basic histopathology data alone.

The aim of this study was to examine the role of MAPK and PI3K/AKT/mTOR signaling in a panel of lapatinib sensitive and resistant HER2-amplified breast cancer cell lines to identify pharmacodynamic markers of response to lapatinib treatment.

Methods: Dose response curves were generated to determine sensitivity to lapatinib in a panel of 17 HER2-amplified breast cancer cell lines. Total and phosphorylated levels of HER2, HER3, EGFR, AKT, ERK, S6K and eEF2 were determined following 24 hours lapatinib treatment in each of the cell lines.

Results: Twelve of the cell lines were sensitive to lapatinib with IC_{50} < 1 μM while 5 of the cell lines were innately resistant to lapatinib with IC_{50} > 1 μM. Levels of pHER2 and pHER3 were decreased in response to lapatinib in all cell lines independent of sensitivity to lapatinib. This suggests that inhibition of HER2/HER3 activation is not indicative of response to lapatinib treatment. There was also no correlation between the levels of HER2, HER3, and EGFR and sensitivity to lapatinib in the cell line panel.

In lapatinib sensitive cell lines, lapatinib decreased PI3K (pAKT), mTOR (pS6K) and MAPK (pERK) signaling and increased pEF2 levels. In contrast the levels of pAKT, pS6K, pEF2 and pERK were maintained following lapatinib treatment in lapatinib resistant cells. The continued activation of these proteins in the presence of lapatinib suggests a possible feedback mechanism that warrants further investigation. These data confirm that maintained signaling through either the PI3K/AKT/mTOR pathway or the MAPK pathways in the presence of lapatinib can be an early pharmacodynamic biomarker of response.

Conclusions: Maintenance of pAKT, pS6K, pEF2 and pERK levels, in response to lapatinib treatment correlates with lapatinib resistance. These data suggest that alterations in the PI3K/AKT/mTOR and MAPK pathways play an important role in innate lapatinib resistance and pharmacologically targeting these pathways is a rational therapeutic approach for overcoming innate lapatinib resistance.
PI-12-07
Pharmacokinetics (PK) of Trastuzumab Emtansine and Paclitaxel or Docetaxel in Patients with HER2-Positive MBC Previously Treated with a Trastuzumab-Containing Regimen.
Lu D, Modi S, Elias AD, Agarwal P, Yi J-H, Guardino AE, Althouse BL, Girish S. Genentech, South San Francisco, CA; Memorial Sloan-Kettering Cancer Center, New York, NY; University of Colorado, Aurora, CO

Background
Trastuzumab emtansine (T-DM1) is an antibody-drug conjugate composed of trastuzumab, DM1 (a potent cytotoxic tubulin polymerization inhibitor), and the stable MCC linker. Based on in vitro and preclinical data suggesting synergy in tumor cell cytotoxicity with T-DM1 + taxanes, these combinations are being tested in phase Ib studies. The assessment of risk for PK-based drug interaction (PK-DI) with these combinations is important. T-DM1 is expected to undergo proteolytic degradation with no significant involvement of cytochrome P450 isoenzymes (CYPs), and DM1 is metabolized mainly by CYP3A4 (and to a lesser extent by CYP2C8). Paclitaxel (P) and docetaxel (D) are metabolized primarily by CYP2C8 and CYP3A4, respectively. None of these drugs inhibits or induces CYPs at clinically relevant concentrations. Since DM1 and D are both metabolized by CYP3A4, PK-DI may occur. This is less likely with DM1 and P since they are primarily metabolized by different CYPs.

Methods
The tolerability, PK, and dose-limiting toxicities of T-DM1 + P or D were investigated in 2 studies (TDM4652g and BP22572) of patients with HER2-positive MBC previously treated with a trastuzumab-containing regimen. For PK-DI analysis, both studies used a staggered dosing design in the first cycle, with same-day dosing in subsequent cycles. Study BP22572 was later amended to give both drugs on the same day in all cycles. Either P (65 mg/m², n=11; 80 mg/m², n=6) or D (60 mg/m², n=12; 75 mg/m², n=6) was combined with T-DM1 at various doses (2 mg/kg or 2.4 mg/kg q3w or 1.2 mg/kg qw for TDM4652g; 2.4 mg/kg or 3.6 mg/kg qw for BP22572). PK of T-DM1, total trastuzumab (conjugated + unconjugated trastuzumab), DM1, P, and D were evaluated and compared with historical single-agent data and across cycles within the study, using population analysis and/or noncompartmental analysis (NCA), as data allowed. Preliminary safety and efficacy data for the historical T-DM1 single-agent studies were reported previously (Krop JCO 2010; Burris JCO 2010; Krop ESMO 2010).

Results
The PK of P and D were similar from cycle 1 to cycle 2 for maximum concentration (Cmax), area under the concentration-time curve, half-life, clearance (CL) and steady-state volume of distribution, and were also similar to historical single-agent data. The Cmax of DM1 was ~8 ng/mL in both studies, and the average Cmax values were similar to historical single-agent data. T-DM1 PK parameters for the combination studies are within the range of historical single-agent data (Table). Data from additional patients and doses in the combination studies will be presented.

Bayesian post hoc PK parameters for T-DM1

<table>
<thead>
<tr>
<th>Data source</th>
<th>Patients, n</th>
<th>CL (L/day), median [range]</th>
<th>Vf (L), median [range]</th>
</tr>
</thead>
<tbody>
<tr>
<td>BP22572 (TDM1 + D)</td>
<td>18</td>
<td>0.542 [0.413–0.671]</td>
<td>2.41 [1.98–3.03]</td>
</tr>
<tr>
<td>TDM4652g (T-DM1 + P)</td>
<td>17</td>
<td>0.768 [0.526–1.16]</td>
<td>3.15 [2.18–4.07]</td>
</tr>
<tr>
<td>Historical data (T-DM1)</td>
<td>168</td>
<td>0.609 [0.296–1.28]</td>
<td>2.27 [1.90–5.21]</td>
</tr>
</tbody>
</table>

Conclusion
The risk of clinically relevant PK-DI between T-DM1 and P or D appears to be low.
P1-12-09
Safety and Efficacy of Neratinib in Combination with Capecitabine in Patients with ErbB2-Positive Breast Cancer.
Saura C, Garcia-Saenz JA, Xu B, Harb W, Moroose R, Phuard T, Kiger C, Germa C, Wang K, Kim S-B. Breast Cancer Unit, Vall d’Hebron University Hospital, Barcelona, Spain; Hospital Clínico San Carlos, Madrid, Spain; Cancer Hospital, Chinese Academy of Medical Sciences, Beijing, China; Horizon Oncology Center, Lafayette, IN; Florida Hospital Cancer Institute, Orlando, FL; Washington University School of Medicine, St. Louis, MO; Pfizer Global Research and Development, Paris, France; Pfizer Inc, Pearl River, NY; Asan Medical Center, Seoul, Korea

Background: Neratinib (HKI-272) is an irreversible pan-ErbB receptor tyrosine kinase inhibitor that has shown antitumor activity in patients with ErbB2+ breast cancer. Capecitabine has demonstrated efficacy and tolerability in combination with lapatinib, a reversible dual ErbB1/ErbB2 kinase inhibitor, in patients with ErbB2+ advanced breast cancer. The current study evaluated the safety and clinical activity of neratinib in combination with capecitabine.

Methods: In part 1 of this open-label, phase 1/2 study, the maximum tolerated dose (MTD) of neratinib in combination with capecitabine was determined in adults with advanced solid tumors. Part 2 of the study further evaluated the safety and clinical activity of neratinib plus capecitabine at the MTD in adults with confirmed ErbB2+ metastatic or locally advanced breast cancer (ECOG Performance Status of 0-2). Eligible patients had received prior taxane treatment and ≥1 prior trastuzumab-containing regimen for ≥6 weeks for metastatic or locally advanced disease. The primary endpoint of part 2 was objective response rate (ORR); tumor responses were assessed by investigators using modified RECIST version 1.0 guidelines every 6 weeks.

Results: In part 1 (n = 33), the MTD was determined to be neratinib 240 mg/day plus capecitabine 750 mg/m² twice daily on Days 1 to 14 of each 21-day cycle. In part 2, as of April 2011, 72 female patients (median age of 52 years [range, 33-79 years]) with ErbB2+ breast cancer were enrolled and treated at the MTD; 7 patients had prior lapatinib exposure and all had prior trastuzumab and taxane exposure. As of the snapshot date, 56% of patients at the MTD were still participating in the study. The most common drug-related adverse events (AEs) in part 2 were diarrhea (89%), palmar-plantar erythrodysesthesia (57%), nausea (33%), vomiting (26%), and decreased appetite (22%). Grade 3/4 drug-related AEs in ≥25% of patients were diarrhea (25%) and palmar-plantar erythrodysesthesia (13%). Eight patients withdrew from part 2 due to AEs, including 4 who withdrew due to diarrhea. Dose interruptions of neratinib and capecitabine, respectively, due to AEs were required by 19 and 22 patients; dose reductions due to AEs were required by 8 and 22 patients. As of June 2010 (interim analysis), 22 patients were evaluable for efficacy in part 2 of the study. Of these 22 patients, 11 achieved a partial response for an ORR of 50%. An additional 2 patients maintained stable disease for ≥24 weeks, resulting in a clinical benefit rate of 59%, and 8 patients had stable disease for <24 weeks. One patient had progressive disease without achieving a response or stable disease. Updated efficacy data will be presented.

Conclusions: The results of this study indicate that neratinib combined with capecitabine is tolerable and has promising antitumor activity in patients with ErbB2+ metastatic or locally advanced breast cancer pretreated with trastuzumab. This study supports further evaluation of this combination in ErbB2+ breast cancer.

P1-12-10
Phase II Study Evaluating Lapatinib (L) in Combination with Albumin Bound Paclitaxel (ab-Pac) in Women Who Have Received 0-1 Chemotherapy Regimen for HER2 Overexpressing (HER2+) Metastatic Breast Cancer (MBC).
Yardley DA, Hart L, Bosserman L, Saleh MN, Waterhouse DM, Richards P, Hagan MK, DeSilvio ML, Mahoney JM, Nagarwala Y. Sarah Cannon Research Institute; Tennessee Oncology, PLLC; Florida Cancer Specialists; Willshire Oncology Medical Group; Georgia Cancer Specialists; Oncology & Hematology Care, Inc.; Virginia Cancer Care; Oncology & Hematology Associates of SW; GlaxoSmithKline, Collegeville, PA

Background: L, a dual kinase inhibitor of epidermal growth factor receptor (EGFR) and the human epidermal growth factor receptor-2 (HER2), approved for the treatment of HER2+ MBC in combination with capecitabine following progression after trastuzumab, anthracycline, and taxane. L in combination with chemotherapy has significantly improved progression free survival in patients (pts) with HER2+ MBC. Ab-Pac is a cremophor free, albumin-bound paclitaxel approved for use in pts with MBC demonstrating superior efficacy and safety when compared to other taxanes.

Methods: Phase II study (LPT111111) evaluated the efficacy and safety of L in combination with ab-Pac in 60 pts with histologically confirmed stage IV HER2+ (IHC 3+/FISH+) invasive MBC. Pts received 0-1 prior chemotherapy regimen in the metastatic setting and no prior treatment with L. Prior taxane therapy permitted provided this was ≥12 months prior to study entry, LVEF>50%, peripheral neuropathy <2, prior CNS mets permitted, and prior endocrine therapy permitted. Pts received ab-Pac (125 mg/m² IV on Days 1, 8, 15, q28 days) plus L (1250 mg daily). Planned safety analysis of the first 5 pts prompted a protocol amendment with a 20% dose reduction for both agents due to Grade (G) 3 neurotoxicity and diarrhea. Subsequent pts received ab-Pac (100 mg/m² IV on Day 1, 8, 15, q28 days) in combination with L (1000 mg daily). Pts with SD or a response continued L alone until progression. Response assessments performed every 2 cycles. The primary endpoint was overall response rate (ORR) and secondary endpoints were progression-free survival (PFS), time to response, duration of response and overall survival (OS).

Results: Here we present the final analysis of all subjects receiving at least 6 months of protocol therapy. Median age is 56 years; 45 pts (75%) received treatment as 1st line therapy and 15 (25%) as 2nd line; 57% hormone receptor positive and 43% negative; 42% received trastuzumab and 40% received a taxane in either (neo) adjuvant or metastatic setting. After a median of 5.6 months, 7% pts had a complete response, 47% a partial response and 17% had stable disease, the ORR was 53% [95% CI: 41% to 66%]. The median time to response = 7.8 wks [95% CI: 7.4 to 8.1] with a median duration of response of 48.7 wks [95% CI: 31.7 to 57.1]. The median PFS was 39.7 wks [95% CI: 34.1 to 63.9]. Duration of exposure to ab-Pac; 48% received less than 6 cycles, 30% received 6 cycles and 22% received greater than 6 cycles. Table 1 shows the most common G ≥2 treatment-related toxicities.

<table>
<thead>
<tr>
<th>Adverse Events</th>
<th>Grade 2 (%)</th>
<th>Grade 3 (%)</th>
<th>Grade 4 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhea</td>
<td>21</td>
<td>20</td>
<td>4</td>
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<tr>
<td>Fatigue</td>
<td>10</td>
<td>8</td>
<td>2</td>
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<tr>
<td>Nausea</td>
<td>22</td>
<td>22</td>
<td>2</td>
</tr>
<tr>
<td>Rash</td>
<td>18</td>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>10</td>
<td>22</td>
<td>0</td>
</tr>
</tbody>
</table>

Two fatal adverse events; one pt with a h/o arrhythmia experienced sudden death of presumed cardiac origin and the other subject with h/o COPD, hypertension and uncontrolled diabetes experienced acute renal failure. No G 3/4 elevation in LFTs observed.
Conclusions: L 1000 mg with ab-Pac 100 mg/m² IV on Day 1, 8, 15, q28 day is feasible with manageable and predictable toxicity. The ORR of 53% compares favorably with other HER2 based combinations in this setting and warrants further exploration.

P1-12-11
Neoadjuvant Chemotherapy-Trastuzumab Versus Neoadjuvant Chemotherapy Followed by Post-Operative Trastuzumab: A Multicentre Study.

Palmieri C, Yan K, Oswaldly W, Shah D, Gajis O, North B, Riddle P, Ahmad R, Lewanski C, Coombes RC, Cleator S, Howell S, Beresford M. Imperial College Healthcare NHD Trust, London, United Kingdom; Bristol Haematology and Oncology Centre, Bristol, United Kingdom; Third Faculty of Medicine, Charles University, Prague, Czech Republic; Imperial College London, London, United Kingdom; The Christie NHS Foundation Trust, Manchester, United Kingdom

Background: The addition of neoadjuvant trastuzumab (NAT) to neoadjuvant chemotherapy (NCT) has been shown to increase the rate of breast preservation and pathological complete response (pCR) compared to neoadjuvant chemotherapy alone (NCT). Furthermore, pCR following NCT-NAT is higher and pCR in this setting has been associated with improved disease free survival (DFS) compared to NCT alone group. No study has yet investigated the effect of NCT-NAT versus NCT followed by surgery followed by adjuvant trastuzumab (AT) on disease free survival and overall survival.

Methods: All cases of invasive breast cancer diagnosed at Imperial College Healthcare NHS Trust, Christie Hospital and University Hospitals Bristol NHS Foundation Trust between 2006-2010 were reviewed and all early HER2 positive breast cancers receiving neoadjuvant treatment were identified, and case notes reviewed. Histopathological details, neoadjuvant treatment and where trastuzumab was initiated were recorded. pCR rate was documented and an efficacy analysis performed, disease free survival (DFS) and overall survival (OS) for the whole cohort, and for cases treated with NCT-NAT versus NCT alone followed by surgery and AT.

Results: Between 2006-2010 122 invasive HER2 positive invasive breast cancers were treated in the neoadjuvant setting at the 3 centres. Of these 58% (71) received NAT alone and 42% (51) were treated with NCT-NAT. The median follow up for whole group was 26 months (range 4.9-70.4), 30.5 months (range 10.5-70.4) for NCT group and 20.1 months (range 4.9-59) for NCT-NAT group. 19 (27%) relapse/death events in NCT group compared to 7 (14%) in NCT-NAT group to 7 (14%) in NCT-NAT group. The median follow up for whole group was 26 months.

Conclusion: Neoadjuvant chemotherapy-Trastuzumab is associated with better DFS and OS compared to Neoadjuvant chemotherapy followed by Post-Operative Trastuzumab.
vs trastuzumab + docetaxel was investigated in patients with no prior MBC treatment in the randomized phase II study TDM4450g/BO21976. Here we report the PK of T-DM1 from that study and compare these data with those from studies that enrolled pretreated patients.

Methods

In all 3 studies, PK parameters, including maximum concentration (Cmax), area under the concentration-time curve (AUC), terminal half-life (t1/2), steady-state volume of distribution (Vss), and clearance (CL) were estimated by noncompartmental analysis (NCA) for serum T-DM1, serum total trastuzumab (conjugated and unconjugated), and plasma DM1. The effects of baseline trastuzumab and HER2 extracellular domain (ECD) concentration on T-DM1 exposure were explored and the relationship between T-DM1 exposure and clinical response (objective response rate [ORR] and progression-free survival [PFS]) was modeled.

Results

T-DM1 PK from evaluable patients enrolled in 3 studies are shown in Table 1. No significant correlations were observed between efficacy (as measured by ORR) and T-DM1 exposure (AUC, Cmax) after administration of T-DM1 to pretreated patients; efficacy-exposure analyses (ORR and PFS) for previously untreated patients will be presented. Patients with measurable concentrations of trastuzumab at baseline had a greater AUC during cycle 1; however, this did not impact ORR. Baseline circulating HER2 ECD concentrations also had no effect on ORR for pretreated patients. The impact of baseline trastuzumab and HER2 ECD concentrations on ORR and PFS in previously untreated patients will be presented.

PK of T-DM1 3.6 mg/kg q3w in 3 phase II studies

<table>
<thead>
<tr>
<th>Study (PK evaluable n, total N)</th>
<th>Serum total trastuzumab</th>
<th>Plasma DM1</th>
<th>T-DM1</th>
<th>Cmax (μg/mL)</th>
<th>Cmax (ng/mL)</th>
<th>Cmax (μg/mL)</th>
<th>Cmax (ng/mL)</th>
<th>C(0→inf) (μg/mL)</th>
<th>C(0→inf) (ng/mL)</th>
<th>AUC(τ1/2) (μg/mL)</th>
<th>Vss (mL/kg)</th>
<th>CL (mL/day/kg)</th>
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<tbody>
<tr>
<td>TDM4450g—no prior MBC treatment</td>
<td>81.5 (20.6)</td>
<td>5.0 (2.41)</td>
<td>84.9</td>
<td>30.6</td>
<td>476 (119)</td>
<td>3.58 (0.666)</td>
<td>30.0 (8.21)</td>
<td>8.09 (2.50)</td>
<td></td>
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</tr>
<tr>
<td>TDM4450g—pretreated</td>
<td>88.0 (30.2)</td>
<td>5.3 (2.03)</td>
<td>80.9</td>
<td>20.7</td>
<td>457 (129)</td>
<td>3.53 (0.714)</td>
<td>28.4 (12.9)</td>
<td>8.51 (2.69)</td>
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</tr>
<tr>
<td>TDM4450g—pretreated* (105; 110)</td>
<td>89.9 (31.3)</td>
<td>5.36 (2.56)</td>
<td>79.5</td>
<td>21.1</td>
<td>486 (141)</td>
<td>3.96 (0.964)</td>
<td>31.2 (10.9)</td>
<td>8.04 (2.97)</td>
<td></td>
<td></td>
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</tbody>
</table>

*Median number of non-hormonal therapies for MBC was 5 (range 1–17) in TDM4258g and 7 (range 3–17) in TDM4374g

Conclusions

Single-agent T-DM1 has similar PK in patients who have received prior therapy for MBC and in those who have not. The PK of T-DM1 was not affected by prior trastuzumab treatment or by circulating HER2 ECD, and no significant correlations were observed between efficacy (ORR) and T-DM1 exposure (AUC, Cmax) or HER2 ECD for pretreated patients. The relationships between efficacy and T-DM1 exposure and HER2 ECD concentrations will be presented for patients without prior MBC treatment.

PI-12-14

Genetic Ablation or Pharmacological Inhibition of Autophagy Suppresses Intrinsic Resistance of Breast Cancer to HER2-Targeted Therapies.

Cufi S, Oliveras-Ferraros C, Vazquez-Martin A, Sauri-Nadal T, Del Barco S, Martin-Castillo B, Lopez-Bonet E, Menendez JA. Catalan Institute of Oncology, Girona, Catalonia, Spain; Girona Biomedical Research Institute (IDIBGI), Girona, Catalonia, Spain; University Hospital of Girona Dr. Josep Trueta, Girona, Catalonia, Spain

Autophagy, an evolutionary conserved catabolic process whereby cells generate energy and building blocks by promoting large-scale recycling of cytoplasmic macromolecules and organelles, represents a novel drug-targetable molecular mechanism underlying de novo (primary) refractoriness of HER2 gene-amplified breast cancer (BC) to HER2 inhibition. JIMT-1 cells, which were obtained from a HER2-positive BC patient that rapidly progressed on trastuzumab (Herceptin™) ab initio and that show cross-resistance to multiple HER1/2 inhibiting drugs including lapatinib (Tykerb™), were found to constitutively exhibit an enhanced autophagic vesicle content as assessed by immunoblotting of endogenous ATG8/LC3 lipids and confocal imaging of the recruitment of ATG8/LC3 to autophagic vesicles. A significant decrease in the expression status of the specific autophagy receptor p62/SQSTM1—a protein selectively degraded by autophagy—confirmed further a constitutive activation of the autophagic flux in trastuzumab-refractory JIMT-1 cells. When the Human Autophagy RT Profiler™ PCR Array was employed to profile the expression of 84 genes involved in autophagy, ATG12 was the most differentially up-regulated gene in JIMT-1 cells (>10-fold) as compared with trastuzumab-responsive SKBR3 cells. Upon collection of the transcriptional profile of the ATG12 gene across two sets of >50 widely used BC cell lines, HER2+ BC cells with well-established de novo resistance to trastuzumab were characterized by expressing significantly higher levels of ATG12. When lentiviral-delivered small hairpin RNA was employed to stably & specifically knock-down ATG12 gene, JIMT-1 ATG12-shRNA cells were more significantly growth-inhibited by trastuzumab (up to 5-fold) than parental JIMT-1 cells. Moreover, the half-maximal inhibitory concentration (IC50) values for the small-molecule HER1/2 Tyrosine Kinase Inhibitors gefitinib, erlotinib and lapatinib were drastically reduced by up to 10 times in response to ATG12 gene silencing. Knockdown of autophagy-regulatory genes other than ATG12 (e.g. ATG5, ATG8/ LC3B) similarly suppressed refractoriness to trastuzumab as well as co-existing resistance to other HER1/2-targeted agents. Knockdown of autophagy-specific genes, however, did not alter basal sensitivity of JIMT-1 cells to multiple cytotoxics including doxorubicin, cisplatin, paclitaxel and vinorelbine. When lysosomal degradation was pharmacologically inhibited by using the antimalarial lysosomotropic drug chloroquine we found a massive accumulation of abnormal autophagolysosomes that promoted synergistic re-sensitization of JIMT-1 cells to the growth inhibitory and apoptotic activity of trastuzumab. In summary, autophagy-specific genes appear to play a potent protective role against HER2 inhibiting drugs currently in use. Given that genetic and pharmacological targeting of autophagy was found to be detrimental to intrinsic BC refractoriness to HER2-targeted therapies, our current findings may provide rationale for novel, chloroquine-based therapeutic approaches aimed to circumvent primary-resistance and potentiate the efficacy of both trastuzumab and lapatinib in patients treated for HER2-positive BC disease.
**PI-12-15**

**Adjuvant Trastuzumab Effect on HER2-Positive Breast Cancers According to Hormonal Receptor (HR) Status: Crossover between ER and EGFR/HER2 Pathway May Prevent Trastuzumab from Improving Outcomes in HER2-Positive and HR-Positive Breast Cancers.**

Park YH, Cho EY, Lee JE, Nam SJ, Yang JH, Ahn JS, Im Y-H. Samsung Medical Center

**Background:** Crossover between growth factor receptor, especially the EGFR/HER2 pathway, and ER pathways has been associated with endocrine resistance. Thus, combination therapy targeting both ER and EGFR/HER2 signaling to block the crosstalk between these pathways and eliminate escape routes have been proven effective in both preclinical and clinical models. Anti-HER2 directed therapy has been reported to restore hormone sensitivity in HER2-positive breast cancers. Adding trastuzumab to conventional treatment has been a standard treatment of choice in HER2-positive breast cancer irrespective of hormonal receptor (HR) status. The purpose of the study is to evaluate adding effect of 1 year of trastuzumab to conventional adjuvant treatment in patients with HER2-positive breast cancer who received surgery according to HR status.

**Patients and Methods:** We retrospectively analyzed the clinicopathologic characteristics and outcomes of 618 postoperative HER2-positive breast cancer patients between 2001 and 2008 at the Samsung Medical Center. Most of HER2-positive patients in our institute were treated with 1 year of trastuzumab as a part of adjuvant therapy since 2007 (post-trastuzumab era) compared with 2000-2006 (pre-trastuzumab era). Clinical outcomes including recurrence-free survival (RFS) were analyzed between pre-trastuzumab and post-trastuzumab era according to HR status. We performed Cox regression multivariate analysis for relapse using variables from univariate analysis by log-rank test for relapse. Clinical presentations and clinicopathologic characteristics were evaluated at the time of recurrence between both eras.

**Results:** The median age at diagnosis was 46 years (range, 22-79). During the median 60.0 months of follow-up, the 5-year recurrence rate was 20.2%. The 618 patients were divided into two groups (patients who received (n=175) and did not receive (n=443) adjuvant trastuzumab). Recurrence rate was much lower in post-trastuzumab era than in pre-trastuzumab era (13.6% vs. 32.3%, p<0.0001). Improving outcomes due to adding trastuzumab in patients with HER2+ve/HR-ve patients showed significant benefit from trastuzumab throughout the follow-up period (p=0.004). However, this improving effect appeared not to be consistent with statistical significance in HER2+ve/HR+ve patients (p=0.135). The analyses were performed according to quantitative ER Allred scores in HER2+ve/ER+ve patients, the effect of adding trastuzumab appeared to be mitigated as time over without any statistical significance (p=0.975). Young age (≤35) (hazard ratio (HR) 2.4, p<0.0001), trastuzumab use (HR 0.4, p=0.001), and node positivity (HR 2.8, p=0.002) were identified as independent prognostic factors for recurrence in Cox-regression multivariate analysis. Limiting to HER2+ve/ER+ve patients, the statistical significances of trastuzumab use as independent factors were not maintained in Cox-regression models (p=0.074 for trastuzumab use).

**Conclusion:** Cross-talk between ER and EGFR/HER2 pathways may mitigate trastuzumab effect in HER2+ve/ER+ve breast cancers. Further study is warranted.

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**PI-12-16**

**HER-2 Testing and Treatment – Is Age a Factor?**

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**Aims:** Elderly breast cancer patients have a poorer prognosis due to late diagnosis and sub-optimal treatment. In the UK, HER-2 testing was introduced in 2005 and we sought to determine whether HER-2 testing was performed on patients of all ages and whether HER-2 positive patients of all ages subsequently received Herceptin, from implementation in 2005 until 2008.

**Methods:** Review of all newly diagnosed breast cancers in women over 50 in our Unit between January 2005 and December 2008 was performed. Cases were identified from the hospital pathology electronic database. Patients were considered HER-2 positive if tissue sections scored 3+ on immunohistochemical analysis (intense membranous staining) or if gene amplification was detected using fluorescence in-situ hybridisation (FISH). Patients were eligible for treatment with Herceptin if the tumour exceeded 1cm in size or if they had lymph-node positive disease, per UK guidelines. Herceptin treatment was determined for all patients.

**Results:** In total 703 patients with a median age of 68 years (range 56 to 98) were identified and 628 (89.3%) underwent definitive surgery (mastectomies: 246; wide local excisions: 382) and axillary surgery. In total 371 patients (52.8%) underwent HER-2 testing by immunohistochemistry or FISH. HER-2 tests performed by year: 2005 (51/131; 38.9%); 2006 (59/187; 31.6%); 2007 (71/189; 37.6%) and 2008 (190/196; 96.9%). Fifty six patients (15.1%) were HER-2 positive by immunohistochemistry or FISH. Median age of HER2 positive patients was 66 years (range 57 to 98); median age of HER2 negative patients was 68 years (range 56 to 96). Forty-four HER2 positive patients (78.6%) were eligible for treatment with Herceptin; of these 28 patients (63.6%) were treated with Herceptin. HER2 testing, treatment with Herceptin and overall 5-year mortality rate for patients between 50 to 69 years and patients aged 70 years or older is summarised in Table1.

**Summary of HER-2 testing, treatment with Herceptin and overall 5-year mortality rate for patients aged between 50 to 69 years and patients aged 70 years or older**

<table>
<thead>
<tr>
<th>Age group</th>
<th>n</th>
<th>n tested</th>
<th>n HER-2 positive</th>
<th>n eligible for Herceptin</th>
<th>n treated with Herceptin</th>
<th>Overall 5-year mortality rate / mortality rate of HER2 positive pts</th>
</tr>
</thead>
<tbody>
<tr>
<td>50-69</td>
<td>1244 (61.7%)</td>
<td>1037 (66.9%)</td>
<td>37 (15.0%)</td>
<td>10 (18.1%)</td>
<td>24 (48.0%)</td>
<td>5% / 13%</td>
</tr>
<tr>
<td>70-84</td>
<td>259 (13.3%)</td>
<td>124 (46.1%)</td>
<td>19 (15.3%)</td>
<td>14 (35.7%)</td>
<td>8 (57.1%)</td>
<td>12% / 29%</td>
</tr>
</tbody>
</table>

n: number of patients; p: patients; CI: confidence interval

Twenty percent of patients aged between 50 to 69 years and 71.4% of patients aged 70 years or older eligible for Herceptin were not treated. Reasons for non-treatment included: multiple co-morbidities (4); treatment declined by oncologists (1); no referral to oncologists (1); death prior to treatment (2) and unknown (8). Median follow up time was 43.3 months (range 0.2 to 73.8). All cause mortality was higher in the over 70 year old breast cancer group (Log Rank Test p<0.001) regardless of HER-2 status.

**Conclusions:** National introduction of routine HER-2 testing for all newly diagnosed breast cancers led to a testing rate increase to almost
treatments warrants further investigation.

The predictive impact of the former method on response to anti-HER2 therapy has been assessed. However, the number of gene copies/nucleus appears to represent polysomy of chromosome 17, it can also be a result of gains of 17q with centromere involvement, or amplification of the centromeric region. The classification of HER2-positive tumors according to the HER2/CEP17 ratio may therefore misclassify a fraction of truly amplified cases as polysomic. We prospectively evaluated tumors with chromosome 17 polysomy but no HER2 amplification to assess HER2 status using the above two FISH classifications and immunohistochemistry (IHC).

**Materials and methods:** Tumors were tested for gene amplification by FISH with probes to HER2/neu and CEP17 using the PathVysion HER-2 DNA Probe Kit (Vysis). Classification was based on the HER2/CEP17 ratio (amplified when > 2.2) and average HER2 gene copy number/nucleus (amplified when > 6 copies). Both polysomic and equivocal cases (HER2/CEP17 ratio 1.8 – 2.2) were further studied by IHC using the HercepTest (Dako) with 0-3 scoring system (overexpression when 3+).

**Results:** From March 2010 to May 2011 we evaluated 31 primary breast cancers showing chromosome 17 polysomy. Median HER2/CEP17 ratio was 1.3 (range 0.5–1.9), median HER2 copy number was 5.4 (range 2.6–13.8), and median CEP17 copy number was 4.2 (range 3.2–8.0). Thirteen (42%) had an average HER2 gene copy number > 6/nucleus (median 6.8, range 6.1–13.8) and would therefore be considered as amplified if classified according to the absolute HER2 gene copy number. Nine (75%) of these were 3+ at IHC and the remaining 4 were 2+, whereas among the 18 cases with an average HER2 gene copy number ≤ 6/nucleus, one was 2+, ten were 1+, and 7 scored 0. Twenty-nine cases showed negative HER2/CEP17 ratios (< 1.8) and three cases equivocal HER2/CEP17 ratios (between 1.8 and 2.2). Using HER2/CEP17 ratio as first assessment and IHC only in equivocal cases, only one of the 31 polysomic cases would have been classified as HER2-positive. However, 8 polysomic cases with HER2/CEP17 ratio < 1.8 showed 3+ immunostaining (all with average HER2 gene copy number > 6/nucleus), while other cases had an average HER2 gene copy number > 6/nucleus with 2+ immunostaining.

**Conclusions:** Our results show that both FISH evaluation criteria and IHC can modify the percentage of polysomic tumors classified as HER2-positive. However, the number of gene copies/nucleus appears more frequently associated with 3+ IHC than the HER2/CEP17 ratio. The predictive impact of the former method on response to anti-HER2 treatments warrants further investigation.

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**P1-12-18**

**Change in HER2 Status in HER2 Positive Operable Breast Cancer Patients Treated with Neoadjuvant Chemotherapy with or without Anti-HER2 Therapy: Analysis of Two Consecutive Cohorts.**

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**Introduction:** Emerging literature data have shown a change of HER2 expression from primary tumors to metastatic deposits. Tumor heterogeneity, genetic drift as well as selective pressure of adjuvant therapy have been suggested to explain this phenomenon. Aim of the present analysis is to evaluate the change in HER2 expression after neoadjuvant chemotherapy with or without anti-HER2 agents.

**Methods:** Two consecutive cohorts of HER2+ breast cancer patients treated with neoadjuvant therapy were identified from a prospectively maintained database including 310 patients. The first cohort (A) includes 38 patients enrolled before 2005, treated with chemotherapy alone. The second cohort (B) includes 48 patients treated with neoadjuvant chemotherapy in combination with antiHER2 agents (trastuzumab or lapatinib).

HER2 expression was evaluated by IHC on pre-treatment core biopsy (tru-cut with 14 gauge needle) and on surgical specimen after neoadjuvant therapy. FISH analysis was performed on IHC 2+ samples.

**Results:** The two cohorts were balanced in respect of tumor stage, patient age, and HR expression. In particular, a co-expression of HER2 and HR was observed in 60% of the patients in cohort A and in 70% of the patients in cohort B (p=0.2). Patients in cohort B have a significantly higher rate of pathologic complete response (pCR) in comparison to cohort A (45% vs 11%, p=0.001). A change in HER2 expression from biopsy to post-therapy samples was observed in 39% of the patients in cohort A vs 12% of the patients in cohort B (p=0.02). No patients with pCR have recurred so far vs 25% of the patients with less than pCR (p=0.005). The rate of recurrence was significantly higher for patients experiencing a change in HER2 expression (50% vs 19%, p=0.018).

**Conclusion:** Contrary to our expectations, patients not receiving anti-HER2 therapy as part of neoadjuvant therapy were more likely to have a change in HER2 status vs patients receiving anti-HER2 neoadjuvant therapy. The change in HER2 status has a negative prognostic impact.

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**P1-12-19**

**Phase I Study of Single Agent Trastuzumab Emtansine in Japanese Patients with Human Epidermal Growth Factor Receptor2 (HER2)-Positive Metastatic Breast Cancer (JO22591).**

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**Background:** Trastuzumab emtansine (T-DM1), first-in-class anti-HER2 antibody–drug conjugate (ADC) is under development for the treatment of HER2-positive recurrent locally advanced or metastatic breast cancer (MBC). T-DM1 is composed of: trastuzumab; DM1, an inhibitor of tubulin polymerization derived from maytansine; and the stable MCC linker that conjugates DM1 and trastuzumab. T-DM1 has been evaluated at multiple dose levels in a phase 1 trial (TDM3569g): every 3 weeks (q3w) (0.3–4.8 mg/kg) and weekly (1.2–2.9 mg/kg), and in two subsequent phase II trials (TDM4258g and TDM4374g).
Methods
This Japanese Phase I study was a single-arm, dose-escalation study in patients with HER2-positive MBC who had received prior therapies that included trastuzumab. The objective of the study was to determine the MTD of T-DM1 during Cycle 1, using the continual reassessment method, among three dose cohorts when administered as a single agent and to investigate safety, tolerability and pharmacokinetics of T-DM1 in patients with HER2-positive MBC. Eligibility criteria were standard for this type of study. T-DM1 was administered every 3 weeks at a dose level of 1.8 mg/kg, 2.4 mg/kg or 3.6 mg/kg. Outcomes were assessed by standard-tumor phase 1 methods. Adverse events were reported using CTCAE version 3.0, and tumor response was assessed according to RECIST version 1.0.

Results
Ten patients were recruited: (1.8 mg/kg [n=1], 2.4 mg/kg [n=4], or 3.6 mg/kg [n=5]). One patient in the 2.4 mg/kg group experienced DLTs (Grade 3 AST increase and ALT increase). No other adverse events corresponding to a DLT were observed in any other patients during the DLT observation period. As a result, the MTD in Japanese MBC patients was determined to be 3.6 mg/kg q3w.

The most frequently reported adverse events, regardless of whether they were related to the study drug, were nausea, fatigue, arthralgia and pyrexia. The main changes in laboratory test values recorded were platelet count decrease, AST increase and ALT increase. Efficacy was preliminarily assessed with tumor responses, a partial response was observed in two patients. Most of the AEs were mild and manageable. There were no marked differences in any pharmacokinetic parameters for T-DM1, DM1 or total trastuzumab following administration of T-DM1 between the JO22591 study and the two Western studies (TDM3569g and TDM4258g), and no data obtained suggested any ethnic differences.

Conclusions
T-DM1 monotherapy (3.6 mg/kg every 3 weeks) was well-tolerated in Japanese patients. PK and safety in Japanese patients were comparable to PK and safety in the Western population. These results support further clinical studies with T-DM1 in Japanese patients.

P1-12-20
The Safety and Tolerability of Vorinostat in Combination with Lapatinib in Advanced Solid Tumors.
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Background: Lapatinib has been previously shown to markedly decrease cancer stem cells (CSC) in HER2-positive breast cancer. In preclinical models, we have demonstrated that histone deacetylase inhibitors (HDACi) such as vorinostat can induce differentiation and decrease CSC. The combination of vorinostat and lapatinib is synergistic with a combination index of 0.32 (synergism if CI <1). We therefore undertook a pilot study to evaluate the combination of these two drugs in advanced solid tumors.

Method: Patients were eligible if they were: age ≥ 18 years with incurable solid tumors, ECOG PS 0-2, adequate organ function, and no prior exposure to HDACi. The first 3 patients received lapatinib at the dose of 1,250 mg continuous daily and vorinostat 300 mg 4 days on 3 days off. The second dose level with lapatinib 1,250 mg continuous daily and vorinostat 400 mg 4 days on 3 days off were administered in 6 patients. Cycles were repeated every 21 days until disease progression. Echocardiogram and radiologic evaluation were performed every 12 weeks. During the first cycle, pharmacokinetic (PK) evaluation was performed on days 18 and 21.

Results: Nine consented patients (7 with metastatic breast cancer, 1 with non-small cell lung cancer, and 1 with thyroid cancer) have been enrolled with the median age of 52 (range 25-66). Patients received an average of 6 prior treatments (range 2-10). No dose limiting toxicity or drug related death have been observed. Grade 1-2 toxicities including diarrhea, fatigue, muscle cramps and stomatitis were observed. No grade 3 or 4 hepatic, renal or cardiac toxicity were observed (including no QTc prolongation and no significant reduction in the left ventricular ejection fraction). Patients have received the maximum of 7 cycles (median 3 cycles, range 2-7). Response: as of June 2011, 2 patients are still on treatment. Two patients achieved stable disease (triple negative metastatic breast cancer and HER2-positive breast cancer), 6 patients with progressive disease, and 1 patient is too early to evaluate for response. PK analysis will be presented at the time of the meeting.

Conclusions: The combination of vorinostat and lapatinib is tolerable and has some antitumor activity in heavily pretreated advanced solid tumors. A phase II study in HER2-positive metastatic breast cancer is underway with lapatinib 1,250 mg continuous daily and vorinostat 400 mg 4 days on 3 days off.

P1-12-21
Adjuvant Trastuzumab Treatment without Adjuvant Chemotherapy in Early Breast Cancer.
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Background: Trastuzumab (T) is approved in most Western countries for the treatment of early, HER2+ breast cancer (BC) parallel or sequential to adjuvant chemotherapy (CT). Nevertheless, as in metastatic disease, the antibody seems to be used without chemotherapy in a selected group of HER2+ patients (pt). The purpose of this analysis is to characterize this subgroup as well as describe safety and efficacy outcome parameters in this German prospective observation trial.

Methods: At present, 2870 patients (pts) have been enrolled and documented in this ongoing non-interventional study from 270 German centers. At data base closure for this analysis, sufficient documentation was available from 2422 eligible pts.

Results: The proportion of pts receiving T without preceding or concomitant CT (noCT) was 180/2422 (7.4 %). This subgroup was characterized by higher age (median 58 vs. 56 in the CT group, p=0.0026; ≥70 years: 18%/10%), smaller primaries (pT1 49%/43%, p=0.11), more favorable grading (G3 45%/53%, p=0.045), a higher proportion of positive hormone receptor (67%/61%, p=0.096) and less radiotherapy (64%/79%, p<0.0001). The strong association to radiotherapy is probably due to the fact, that the choice of this additional modality reflects the overall risk assessment of the pt.
In contrast, there was no difference in nodal involvement (pN0: 52%/51%; positive nodes: mean 2.5/2.4). In multivariate analysis (logistic regression model, not incorporating radiotherapy), hormone receptor status is not predictive (p=0.41), while age ≥65 (p=0.0011), grade 1/2 (p=0.046) and pT1 (p=0.089) independently remain at least borderline significant. The mean number of T administrations was 18 and the median duration of T therapy was 12 months in the noCT group as well as the CT group. 57% of the noCT pts received adjuvant endocrine therapy. In the CT group, 81% received anthracyclines and 61% taxanes.

Based on a still low number of only 13 observed events in the noCT group, no differences in relapse-free survival could be detected (p=0.38). Pathological cardiac findings in the pt’s history was not predictive of therapy selection (6%/7%) and were also similarly distributed at the end of T therapy (7%/8%). Cardiac function disorders of CTC grade 3/4 were reported in 1% of both groups, across all grades slightly less frequent in the noCT group (2%/4%).

**Conclusions:** A small, but distinct group of early BC pts without adjuvant chemotherapy receives T treatment for HER2+ disease. These patients are characterized by higher age and favorable primary tumor staging and grading, but not by cardiac comorbidities.

**P1-12-22**

Impact on Survival of the Level of HER2/neu Gene Amplification in Patients with HER2-Positive (HER2+) Advanced Breast Cancer (AdBrCa) Treated with Trastuzumab (H).

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**Background** The level of HER2/neu (HER2) amplification, defined as HER2/centromeric region of chromosome 17 (CEP17) ratio at dual-color fluorescent in-situ hybridization (FISH) test presents wide variations in clinical practice but its clinical significance is still undefined. We designed this retrospective study to investigate the correlations between level of HER2 amplification in primary (T) and/or metastases (M) and outcome in a cohort of HER2+ AdvBrCa pts treated with a H-containing therapy. **Methods** Retrospective multicentric study designed according to REporting recommendations for tumor MARKer prognostic studies (REMARK). To be included pts must have all the following: metastatic or locally advanced (not amenable of curative surgery) BrCa, HER2+ tumour defined as score 3+ at immunohistochemistry or FISH positive, treatment with a H-containing regimen (no H in neo- or adjuvant setting was allowed), at least one tumour sample from T and/or M available, measurable or evaluable disease, adequate follow up (FU) information. Outcome parameters included event-free survival (EFS) and overall survival (OS). All FISH tests were prospectively performed in a central laboratory of cytogenetic specifically for this study and in accordance with the international guidelines on FISH testing. **Results** Ninety-one females were identified and 63 included in the final analysis. Forty-seven pts had one specimen available from T or M, 16 pts had two specimens from either T and a corresponding M. All M samples were obtained before treatment with H. Median FU time is 19.2 months (range 1.2-94.6). In 11 out of 16 cases (69%) with two tumour specimens HER2/CEP17 ratio was higher in M than in T with a statistically significant difference in the median HER2/CEP17 ratio between T (8.3; range: 3.1-18.4) and the corresponding M (10.9; range: 4.6-20.8) (p=0.004). The incremental gain in HER2/CEP17 ratio was associated with significantly shorter OS after trastuzumab-based therapy (p=0.023). A trend towards a correlation between increase in level of HER2 amplification assessed in M and shorter EFS and OS was observed. No statistically significant correlation was found between level of HER2 amplification assessed in T and EFS and OS. **Conclusions** Level of HER2 amplification reported as HER2/CEP17 ratio is a dynamic parameter in HER2+ AdvBrCa as it increases from T to the matched M in a significant proportion of pts. The gain in HER2 gene copies may represent a prognostic factor for shorter OS when pts with HER2+ AdvBrCa are treated with a H-based therapy. Further studies on the level of HER2 amplification in BrCa metastases are strongly warranted to better understand the biology of HER2-positive breast cancer and to better identify patients with a poorer prognosis after treatment with H.

**P1-12-23**

HER4 Coexpression Is Associated with Improved Recurrence Free Survival in HER2-Positive, Herceptin Treated Patients.

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**Background** Individual therapy efficiency of HER2-positive metastatic and pre-metastatic breast cancer patients varies significantly and spans from effectual responsiveness over acquired insensitivity to complete resistance from the outset. Thus no predictive information can be deduced from HER2 diagnostics so that molecular biomarkers indicative for sensitivity / resistance to Herceptin are needed to be identified. The HER2 related HER4-receptor has been shown to have ambivalent (pro-apoptotic or pro-proliferative) activity and consequently represents a prime candidate to affect HER2 activity under Herceptin treatment. We retrospectively analyzed potential her4 gene amplification and HER4 protein expression in HER2-positive, Herceptin treated patients. Patient’s overall and recurrence free survival was evaluated as a function of HER2/HER4 expression. **Methods:** Using dual color Fluorescence in-situ Hybridization (FISH) probes, Zytovision, Bremerhaven, Germany) and qPCR (LC480, Roche, Penzberg, Germany) we quantitatively investigated primary breast cancer tissues from nearly 50 (FISH) and 160 (PCR) patients who received Herceptin treatment. We quantified the her4 gene copy numbers and evaluated the protein expression profile of all four known HER4 isotypes (JM-a/CYT1, JM-a/CYT2, JM-b/CYT1, JM-b/CYT2). Results: FISH analysis revealed a positive and independent prognostic marker in Herceptin treated breast cancer patients with respect to overall survival. Moreover by quantitative PCR analysis we found a significant variability of HER4 protein expression (JM-a/CYT1 and JM-a/CYT2; no JM-b isotypes) in HER2 positive breast cancer tissues, whereas HER2/HER4 positive patients show a significant better recurrence free survival compared to HER2 positive but HER4 negative patients (p > 0.003). **Conclusions:** HER4 has been demonstrated to potentially exert tumor suppressing activity and in turn to have a favourable impact on the course of breast cancer disease. We show here that HER4 expression prolongs in particular recurrence free survival of Herceptin treated patients which indicates a functional integration of HER4 into anti-HER2 targeting. Complementing functional studies allowing for isotype specific function of HER4 will elucidate the special role of this receptor tyrosine kinase in the context of Herceptin treatment and might facilitate individualized anti-ErbB-receptor targeting with higher efficiency.
P1-12-24
Adherence and Persistence with Lapatinib in Women with Metastatic Breast Cancer Who Were Previously Treated with Trastuzumab.
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Background. Lapatinib is an oral small molecule dual tyrosine kinase inhibitor that binds intracellularly to the ATP binding site of the EGFR and HER2 receptors. In a randomized controlled trial (RCT) of women with HER2+ metastatic breast cancer (MBC) previously treated with trastuzumab, an anthracycline, and taxanes, lapatinib plus capecitabine improved time to progression versus capecitabine monotherapy. In an RCT of postmenopausal women with HR+ MBC, 1st-line therapy with lapatinib plus letrozole improved progression-free survival vs. letrozole monotherapy in the primary analysis HER2+ population. Data on adherence and persistence with lapatinib in typical clinical practice are unavailable.

Methods. This was retrospective observational study of adherence and persistence with lapatinib in women with MBC who were previously treated with trastuzumab. Data were from the Thomson MedStat MarketScan® Commercial and Medicare health insurance claims databases spanning 1/2000-3/2010 and representing >90 million covered lives. Subjects included women with ≥1 claims with a diagnosis of breast cancer, ≥1 claims with a diagnosis of distant metastases, ≥1 trastuzumab claim after first distant metastases diagnosis, and ≥1 lapatinib claim after first trastuzumab claim (date of first lapatinib claim = “index date”). Pts with <12 mos. continuous enrollment pre-index or <3 mos. continuous enrollment post-index were excluded. Measures of lapatinib adherence and persistence were calculated from days supplied information on pharmacy claims and included medication possession ratio (MPR), time to discontinuation (end of supply), time to first treatment interruption (gap during treatment of ≥30 days without supply), and duration of continuous therapy (time to gap of ≥30 days without supply or end of supply).

Results. A total of 1299 pts had ≥1 claims with a diagnosis of breast cancer, ≥1 claims with a diagnosis of distant metastases, ≥1 claims for trastuzumab, and ≥1 claims for lapatinib (1106 Commercial, 93 Medicare). Of these, 666 met all inclusion criteria (572 Commercial, 94 Medicare). Mean (SD) age was 52 (8) years in Commercial pts and 52 (8) years in Medicare pts. Among all pts, 73% had claims history of prior taxanes, 27% prior anthracylines, and 24% prior taxanes and prior anthracylines. Mean (SD) initial lapatinib dosage was 1161 (339) mg daily; 84% initiated lapatinib on 1250 mg daily. Sixty-three percent received index lapatinib in combination with capecitabine; 6% in combination with an aromatase inhibitor. Median follow-up was 12 mos. Mean (SD) number of lapatinib claims was 7.1 (4.9). Mean (SD) MPR was 87% (19%); median MPR was 96%. Kaplan-Meier estimated median time to discontinuation of lapatinib was 7.4 months (95%CI 6.7-8.0 mos). Seventy three percent of patients had no treatment interruption during follow-up. Median duration of continuous therapy was 5.2 mos (95%CI 4.8-5.7 mos).

Conclusions. In women with MBC previously-treated with trastuzumab, adherence and persistence with lapatinib in typical clinical practice are relatively high. Further research is needed to identify predictors and consequences of non-adherence so that efforts may be targeted to enhance adherence to effective treatment.

P1-12-25

Background: In patients with HER-2 over-expressing breast cancer, predictors of trastuzumab response and resistance remain unclear and unvalidated. In an exploratory analysis within a phase II trial, we evaluated various biologic markers as predictors of response or resistance to a trastuzumab containing neoadjuvant chemotherapy regimen.

Methods: A tissue microarray (TMA) was constructed from formalin-fixed paraffin-embedded tumor tissue samples obtained prior to neoadjuvant chemotherapy in 21 (from a total of 30) HER-2 positive stage II-III patients. Protocol treatment consisted of 4 cycles of 5-Fluorouracil, Epirubicin and Cyclophosphamide (FEC\textsubscript{50}) followed by 4 cycles of Docetaxel, Carboplatin (AUC 6) and Trastuzumab (TCH). Immunohistochemical (IHC) analyses of estrogen receptor (ER), progesterone receptor (PR), HER-2, Ki67, EGFR and PTEN were performed utilizing previously published protocols and cut-offs. HER-2 equivocal (2+) by IHC cases was confirmed by prior FISH analysis. Fisher’s exact test was used for statistical analysis.

Results: 21 patients had sufficient tumour for analysis. Median age at diagnosis was 49 years old, 67% were pre-menopausal, and biomarker expression was as follows: 43% ER positive, 29% PR positive, and 100% HER-2 positive. 14 patients (67%) had pathologic complete response (pCR) following the completion of neoadjuvant chemotherapy and trastuzumab. In general, tumour samples showed high Ki67 (>14%) staining (62%), low EGFR positivity (14%), and high PTEN expression (71%). Neither high Ki67 expression (p=0.65), any EGFR expression (p=0.26), nor PTEN loss (p=0.60) was associated with higher or lower rates of pCR respectively.

Conclusion: In our small cohort of locally advanced HER-2 over-expressing tumours, baseline expression of Ki67, EGFR and PTEN were not associated with pCR to a neoadjuvant sequential anthracycline and taxane/trastuzumab combination regimen. Additional biomarkers for an activated PI3K pathway and for p95 will be attempted.

P1-12-26
Global Patterns of Care for HER2/Neu Overexpressing Breast Cancer.

Background: Her2/neu overexpression is an independent adverse prognostic factor present in approximately 25% of invasive breast cancers. HER2-overexpressing breast cancer is particularly common in younger patients and therefore poses a significant public health burden. Anti-Her2/neu adjuvant trastuzumab significantly reduces the risk of recurrence as well as improves survival. In view of the serious potential ramifications of not receiving anti-Her2/neu therapy when appropriate, we undertook this international project to determine clinical access to Her2/neu testing and treatment patterns for women with HER2/neu-positive early breast cancer.
Methods: A web-based survey was sent to 386 physicians from 33 countries who participated in the “TEACH” trial, a double blind placebo-controlled phase III study of a novel anti-Her2/neu therapy, lapatinib, in women with primary Her2/neu-positive breast cancer. The survey contained 27 questions addressing physician and patient demographics, access to Her-2/Neu testing in everyday clinical practice, and anti-Her2/neu treatment options in a variety of clinical scenarios.

Results: One hundred and fifty one participants (39%) from 28 countries answered the survey. Ninety eight percent of the participants reported having Her2/neu tumor expression routinely measured for clinical practice in their institutions by immunohistochemistry (83%), FISH (78%) and other methods (17%). Among Asian physicians, 18% did not have routine testing available and sent primary tumors for central testing for TEACH eligibility. Forty eight percent of physicians surveyed reported instances when they had recommended adjuvant Her2-directed therapy to a patient who eventually did not receive it. The proportion of physicians from developing countries that reported patients not receiving therapy was higher than those from developed countries (68% vs. 38%, respectively). The main reason for not receiving trastuzumab was cost in developing countries, while in more developed countries patient refusal and co-morbidities were the main reasons.

Discussion: This survey reflects availability of HER2 breast tumor testing and anti-Her2/neu therapy among physicians from 28 countries worldwide who participated in an anti-Her2/neu therapy clinical trial of a free anti-Her2/neu therapy. These results indicate that a high proportion of women with Her2/neu-overexpressing breast cancer may not receive standard anti-Her2/neu adjuvant therapy especially in developing countries, the barrier to treatment being cost of therapy. We are extending our access to care survey project to a more unslected diverse group of physicians in developing countries.

P1-13-01
An Update of a Phase II Trial of the HER2 Peptide AE37 Vaccine in Breast Cancer Patients To Prevent Recurrence.
Hale DF, Perez S, Sears AK, Clifton GT, Vreeland TJ, Holmes JP, Mittendorf EA, Patil R, Clive KS, Holmes JP, Mittendorf E, Ponniah S, Ponniah S, Papamichail M, Peoples GE, Anderson Cancer Center, Houston, TX; Saint Savas Cancer Hospital, Athens, Greece; Naval Medical Center San Diego, San Diego, CA; Uniformed Services University of the Health Sciences, USMCI, Bethesda, MD; UT M.D. Anderson Cancer Center, Houston, TX

Introduction: AE37 is a tetra-histidine modified Ii-Key hybrid of the HER2-derived peptide AE36 (HER2:776-790). A phase I trial administering AE37 with the immunoadjuvant GM-CSF demonstrated the vaccine to be safe and capable of stimulating CD4+ helper T-cells with HER2-specific anti-tumor activity. Here we present an update of our prospective, randomized, single-blinded, phase II trial of the AE37 vaccine for the prevention of breast cancer recurrence in disease-free, high risk patients.

Methods: After completion of standard therapy, disease-free, node positive or high risk node negative breast cancer patients were randomized to receive either AE37+GM-CSF (vaccine) or GM-CSF alone (control) in six monthly intradermal inoculations. Patients were enrolled with any level of HER2 expression, (IHC 1+ 2+ or 3+). Specific immunologic responses to both AE36 and AE37 were evaluated in all patients at pre-determined intervals: before (RO), mid-series (R3), upon completion (R6), and at six (RC6) and 12 (RC12) months after completion of the vaccine series. In vitro responses were measured using the [3H]-thymidine incorporation assay and in vivo responses using delayed-type hypersensitivity (DTH) reactions. The trial’s primary endpoint is disease recurrence.

Results: To date, 215 patients have enrolled (vaccine=92, control=123). 99% of local and systemic toxicities were ≤ grade 2 or less. There were no grade 4-5 local or systemic toxicities and no difference between toxicity profiles of vaccine and control groups. Vaccine patients exhibited a statistically significant increase from baseline in AE36 and AE37 proliferative responses at each time point, including maintenance of this response up to 12 months post-vaccination (AE36 (cpm): R0=0, R3=1335, R6=1242, RC6=1586, RC12=1360; AE37: R0=0, R3=2859, R6=2300, RC6=3235, RC12=3279, p<0.001) while there have been no proliferative changes for control patients (AE36: R0=91, R3=95, R6=97, RC6=126, RC12=48; AE37: R0=291, R3=399, R6=319, RC6=103, RC12=0). Vaccine patients also had statistically significant increases in DTH reactions to both AE36 and AE37 (AE36 (mm): R0=0, R6=15, RC6=15, RC12=15; AE37: R0=0, R6=24, RC6=17, RC12=20, p<0.001) while controls had no response (AE36: R0, R6, RC6, RC12=0; AE37: R0, R6, RC6, RC12=0). With a median follow up of 17 months, breast cancer recurrences were reduced by 42% in vaccine patients compared to control patients (7.6% vs. 13.2%, p=0.15). In an analysis of patients with low HER2 expression (IHC 1 or 2+), vaccine patients experienced a 49% reduction in recurrence compared to controls (9.5% vs. 18.6%, p=0.16) with no reduction seen in HER2 over-expressing patients (6.0% vs. 7.9%, p=0.49).

Conclusions: The AE37 vaccine is safe and well tolerated with only mild toxicity, which is attributable to the GM-CSF immunoadjuvant. The AE37 vaccine elicits strong peptide specific in-vivo and ex-vivo immune responses, which are maintained for 12 months after completion of the vaccine series. While the number of recurrences are still low, the recurrence rate appears to decrease in vaccinated patients. Administration of the AE37 vaccine may reduce the risk of breast cancer recurrence with the greatest benefit in patients with low levels of HER2 expression.

P1-13-02
Long-Term Clinical Benefit of Adjuvant Breast Cancer Vaccine: 5 Year Efficacy of E75 with Multiple Booster Inoculations.
Vreeland TJ, Clifton GT, Sears AK, Hale DF, Patil R, Clive KS, Holmes JP, Mittendorf EA, Ponniah S, Peoples GE, Brooke Army Medical Center, Ft. Sam Houston, TX; Windber Medical Center, Windber, PA; Naval Medical Center San Diego, San Diego, CA; UT M.D. Anderson Cancer Center, Houston, TX

Background: We are conducting phase I/II clinical trials vaccinating breast cancer patients with E75, an HLA-A2/A3 restricted HER2/neu (HER2) peptide mixed with GM-CSF. The vaccine has been studied in the adjuvant setting to prevent recurrences in clinically disease-free patients after completion of standard therapy. We have previously reported that the vaccine is safe, effectively stimulates HER2-specific immunity, and appears to improve disease-free survival at 24 months. Here, we report long-term data at a median follow-up of 60 months.

Methods: The phase I/II trials were performed as dose escalation/schedule optimization trials enrolling node positive and high-risk, node-negative patients with tumors expressing any level of HER2. Vaccinated patients were given 4-6 monthly inoculations of E75 with GM-CSF immunoadjuvant. Due to waning immunity, a voluntary
booster program was initiated, with inoculations every 6 months after completion of the primary vaccine series (PVS). Patients were monitored for local and systemic toxicities, which were graded by the NCI’s Common Terminology Criteria for Adverse Events. Vaccinated patients and controls were followed for 60 months and recurrences were documented. Demographic differences were compared with the Fisher’s exact test and survival was analyzed by the log-rank test.

### Results
- 187 patients were enrolled; 108 in the vaccine group (VG) and 79 in the unvaccinated control group (CG). The vaccine and control groups were well-matched with the only statistically significant difference being ER-/PR- status (31.1% in VG vs 17.7% in CG, p=0.04). Vaccination was well tolerated with primarily grade 1 and grade 2 toxicity in the PVS (Local Toxicity: 85% Grade 1, 15% Grade 2, 0% Grade 3; systemic toxicity: 71% Grade 1, 14% Grade 2, and 3% Grade 3). Fifty-three of the VG patients received at least one booster, with 34 receiving a second booster, 25 a third, 22 a fourth, 12 a fifth, and 9 receiving at least six boosters. Booster inoculations were well-tolerated with only grade 1 and 2 local and systemic toxicities. There were delayed urticarial reactions in 7/53 (13%) of the boosted patients occurring at a median of 9 days (5-21 days) after inoculation; these were grade 2 reactions and well-tolerated. After a median follow-up of 60 months, there has been a nonsignificant decrease in recurrences observed in the VG compared to the CG (10.6% vs 20.3%, p=0.098). The hazard ratio is 0.52 in the VG. In patients with immunity maintained with voluntary boosters, there have been even fewer (3.8%) recurrences (p=0.03).

### Conclusions
- The E75 breast cancer vaccine is safe and well-tolerated. With long-term follow-up at 60 months, the E75 vaccine continues to show a strong trend toward preventing breast cancer recurrence in vaccinated patients particularly in patients whose immunity is maintained with booster inoculations. To investigate this further, a phase III trial with prospective boosting is being initiated.

### P1-13-03

**Zoledronic Acid Induces an Immune Response in Breast Cancer Patients through Stimulation of Central Memory and Effector Memory gamma/delta T-Cells.**


### Background
- Zoledronic acid (ZA) in combination with endocrine therapy (ET) and ovarian ablation (OA) reported a DFS advantage in premenopausal women with early stage breast cancer (EBC) in ABCSG-12. Emerging evidence from pre-clinical studies suggests that ZA increases gamma/delta T-cells (GDT), an immune cell population with anti-tumor activity. This study examined immune responses to a single dose of ZA in patients with either EBC or metastatic breast cancer (MBC) through sequential measurement of T-cells and immune regulatory cytokines.

### Methods
- Women with EBC/MBC, both pre- and post-menopausal, and no history of immune dysregulation who were scheduled to receive their first-ever dose of ZA were eligible. Blood was collected for serum and blood mononuclear cells at Day 0 (pre-ZA), Day 1 (18-48 hours post-ZA), Day 7 (Day 5-8 post ZA), and Day 28 (Day 25-32 post-ZA). GDT populations and cytokine responses were assayed using flow cytometry and multi-analyte profiling beads (Luminex), respectively. Relative changes from baseline at days 1, 7, and 18 were quantified using log-ratios and analyzed using the Wilcoxon signed-rank test.

### Results
- 24 patients were enrolled from Oct 2009 to Mar 2011. 75% of pts had MBC, and 25% had EBC, while 75% received ET, 17% received chemo (C), and 8% received other. Following ZA administration, a transient decrease in total GDT (CD3+/Vdelta2+) at Day 1 was seen (p<0.003). This was followed by an increase in both effector (CD3+/Vdelta2+/CD45RA+/CD27-) (P=0.005) and central memory GDT (CD3+/Vdelta2+/CD45RA+/CD27+) (P=0.007) and a decrease in naive GDT (CD3+/Vdelta2+/CD45RA+/CD27+) (P=0.006) at day 7; total GDT unchanged. The change in naive GDT and effector GDT appeared to persist at Day 28. IL-1RA (P=0.003), IL-12 (P=0.0005), MIP-16 (P=0.006), IL-10 (P=0.0002) and MIG (P=0.0006), were increased at Day 1 compared to baseline. By day 28, these cytokine levels returned to baseline except IP-10 which appeared to remain elevated. In this limited patient sample, no differences were observed between patients with EBC vs. MBC, ET vs. C, and pre- vs. post-menopausal.

### Conclusion
- ZA appears to induce a highly significant change in immune effector cells in both EBC and MBC patients receiving ET or C. Mobilization of anti-tumor T-cells with a decrease in total (non-specific) GDT followed by a marked increase post-ZA in specific central and effector memory GDT was seen at day 7. Significant increases in cytokine levels, like those seen in this study including those associated with Th1 responses or cell-mediated immunity, as well as IL-12 levels, have been implicated in direct anti-tumor activity. This apparent cytokine and cellular response to ZA could offer an important biologic mechanism for the anti-cancer activity reported in ABCSG-12. Further studies should be performed to determine which subsets of BC patients might achieve these described immune benefits from ZA.

### P1-13-04

**Phase II Study of Topical Imiquimod and Abraxane for Treatment of Breast Cancer Cutaneous Metastases.**

**Salazar LG, Lu H, Gray H, Higgins D, Childs J, Yushe D, Slota M, Parker S, Disis ML. University of Washington, Seattle, WA**

### Background
- Breast cancer (BC) cutaneous lesions can present as local chest wall recurrence or isolated sites of metastatic disease. Current treatments with full thickness chest wall resection, radiation therapy and chemotherapy are not curative; and have significant morbidity and poor overall response rates. Combining local immunomodulation and systemic chemotherapy may be more effective in treating cutaneous disease. Topical imiquimod (IMQ), a TLR-7 agonist, has shown clinical activity against cutaneous metastasis. Pre-clinical studies have shown IMQ to stimulate Th1 cytokine secretion and up-regulate immune co-stimulatory molecules at the tumor site; resulting in augmented tumor specific T cell immunity and tumor growth inhibition. Use of paclitaxel in BC, has demonstrated immunostimulatory effects of increased serum IFN-γ and enhanced NK/LAK cell activity. Abraxane (albumin-bound paclitaxel) may be used in conjunction with IMQ as steroid pre-treatment is not required. We hypothesize the immune effects of Abraxane may synergize and augment the IMQ anti-tumor effects, resulting in greater clinical response. A phase II single-arm study of chemoimmunotherapy with topical IMQ and Abraxane was initiated to determine its safety and therapeutic efficacy; and examine its effect on augmenting endogenous tumor specific immunity and inducing tumor molecular alterations associated with inhibition of tumor growth and/or common pathways of BC immune escape.

### Materials and Methods
- Up to 15 BC patients with cutaneous lesions no longer amenable to standard therapy are enrolled and receive 3
Results

MBC and no prior chemotherapy for MBC. The primary endpoints and C). Patients were required to have measurable, HER2 negative with bev (15 mg/kg q 3 weeks arm A, 10 mg/kg q 2 weeks arms B and A (8.0 months), overall p=0.065. There were no differences in the median age was 57 (range 29-85), 82% were postmenopausal and 260 mg/m

Background: Nanoparticle albumin-bound paclitaxel (nab-P) is superior to paclitaxel 175 mg/m

Methods: This open-label, phase II study randomized patients (pts) to nab-P at 260 mg/m

Conclusions: Chemoimmunotherapy with topical IMQ and Abraxane is well-tolerated and shows excellent clinical efficacy in treating metastatic cutaneous lesions in heavily pretreated BC patients.

P1-14-01

Randomized Phase II Trial of Weekly vs. q 2-Weekly vs. q 3-Weekly Nanoparticle Albumin-Bound Paclitaxel with Bevacizumab as First-Line Therapy for Metastatic Breast Cancer. Seidman AD, Conlin AK, Bach A, Forero-Torres A, Wright G, Hackney MH, Clawson A, Schofield D, Iglesias J, Hudis CA. Memorial Sloan-Kettering Cancer Center; New York, NY; Providence Cancer Center; Portland, OR; University of Alabama - Birmingham, Birmingham, AL; Florida Cancer Institute, Hudson, FL; Virginia Commonwealth University, Richmond, VA; Celgene Corporation, Summit, NJ

Background: Nanoparticle albumin-bound paclitaxel (nab-P) 260 mg/m² is superior to paclitaxel 175 mg/m² (P) every 3 weeks (Gradishar et al. JCO 2005) in metastatic breast cancer (MBC), and weekly uninterrupted P is more effective than q3wk P in MBC (Seidman et al. JCO 2008). Bevacizumab (bev) nearly doubles response rate and time to progression (TTP) when added to P as 1st line therapy for MBC (Miller et al. NEJM 2007).

Methods: This open-label, phase II study randomized patients (pts) to nab-P at 260 mg/m² q3wk (arm B) vs. 260 mg/m² q 2wk with filgrastim (arm B) vs. 130 mg/m² weekly uninterrupted (arm C), all with bev (15 mg/kg q 3 weeks arm A, 10 mg/kg q 2 weeks arms B and C). Patients were required to have measurable, HER2 negative MBC and no prior chemotherapy for MBC. The primary endpoints were response rate and toxicity.

Results: Of 212 pts randomized, 208 (75 arm A, 54 arm B, 79 arm C) were treated, with balanced demographics and baseline characteristics. The median age was 57 (range 29-85), 82% were postmenopausal and 89% had visceral disease (64% lung, 50% liver). ECOG PS 0-60%, 1-35%, 2-5%. 62% had prior neo-adjuvant or adjuvant chemotherapy for early stage disease: anthracycline: 54%, taxane: 38%. No significant differences in confirmed complete and partial response rates were noted (A: 40%, B: 44%, C: 46%). Median TTP was longer in Arm C (9.0 months) versus both arms B (6.3 months) and A (8.0 months), overall p=0.065. There were no differences in overall survival (Arm A: 21.3 months, Arm B: 19 months, Arm C: 25.3 months). As per protocol-specified stopping rule, arm B was closed early due to an unacceptable safety profile with significantly more grade ≥2 fatigue (B:57%, A: 39%, C:39%, p=0.048) and bone pain (B:19%, A:10%, C:4%, p=0.024). Sensory neuropathy was common; grades 2/3/4: Arm A: 29%/32%/1%, Arm B: 15%/50%/22%, Arm C: 27%/43%/1%. Sensory neuropathy was commonly readily reversible with dose delay and reduction. Febrile neutropenia occurred in <2% of pts in all arms. Arm C patients experienced significantly less arthralgia compared with arms A and B, but dose delays were frequent (86% of pts) on this planned uninterrupted weekly schedule. Bevacizumab-related events were consistent with prior phase III trials of taxane/bev; there were no new safety signals.

Conclusions: Significant and similar antitumor activity was observed in all arms. Weekly nab-P with bev (arm C) resulted in longer TTP. Weekly nab-P with bev (arm C) appears to have the highest therapeutic index, however sensory neuropathy is limiting, suggesting that a 3 week on/1 week off schedule could be preferable and should be studied comparatively.

P1-14-02

Correlation of Circulating Tumor Cells (CTC) and Circulating Endothelial Cells (CEC) with Pathological Complete Response (pCR) during Neoadjuvant Chemotherapy (CT) Combined with Bevacizumab in HER2 Negative Inflammatory Breast Cancer (IBC): Ancillary Study of Phase II Trial BEVERLY I. Pierga J-Y, Bidard F-C, Andre F, Petit T, Dalenc F, Dolozier T, Ronieux G, Bonnettere J, Ferrero J-M, Kerbrat P, Lemonnier J, Viens P, Institut Curie, Paris, France; Institut Gustave Roussy, Villejuif, France; Centre Paul Strauss, Strasbourg, France; Centre Claueldes Regaud, Toulouse, France; Centre Francois Baclesse, Caen, France; Centre Val d’Aurelle, Montpellier, France; Centre Oscar Lambret, Lille, France; Centre Antoine Lacassagne, Nice, France; Centre Eugène Marquis, Rennes, France; Unicancer, Paris, France; Centre Paolo Calmettes, Marseille, France

Background: Prognostic value of CTC detection in blood has been reported in primary breast cancer with a rate around 20% in the neoadjuvant setting. No correlation between CTC and pCR to neoadjuvant CT in operable breast cancer has been reported (Pierga CCR 2008, Riethdorf CCR 2010). Predictive value of CEC for response to anti-angiogenic agents is unclear. Methods: CTC and CEC were detected in 7.5 ml and 4 ml of blood respectively with CellSearch™ System in the neoadjuvant setting of HER2 negative IBC (T4d) patients (pts) enrolled in the phase II multicentre trial BEVERLY I, evaluating bevacizumab (15mg/kg q3w) in combination with sequential neoadjuvant CT of 4 cycles of FEC followed by 4 cycles of Docetaxel. Patients received postoperatively 10 cycles of bevacizumab and hormone therapy if tumor was ER+. Results: From 12/08 to 09/10, 101 pts were included, 96 were evaluable for pCR and 92 for CTC and CEC. Out of 96 pts, 51 (53%) had triple negative breast cancer (TNBC). At baseline, 37 pts out of 92 had ≥1 detectable CTC (40%, 95%CI 30-50%).

At baseline, CTC level was not correlated with CEC level, neither with TNBC nor pCR. A drop in CTC incidence was observed from 12/08 to 09/10, 101 pts were included, 96 were evaluable for pCR and 92 for CTC and CEC. Out of 96 pts, 51 (53%) had triple negative breast cancer (TNBC). At baseline, 37 pts out of 92 had ≥1 detectable CTC (40%, 95%CI 30-50%).
months post surgery) none had pCR (p=0.05 Yates test). There was a significant increase of CEC from baseline to presurgery sample (p <0.001) and a decrease (p=0.04) after tumor removal and end of CT. A higher level of CEC (>20/4ml) before C5 could be associated with a higher probability of pCR (Khi2 test, p=0.003). Conclusions: We observed a high CTC detection rate of 40% in HER2-IBC, including TNBC. CEC levels increased progressively during neoadjuvant treatment and decreased after its interruption. Baseline CTC and CEC levels were not predictive of pCR. Detection of CTC at 8 months of follow-up was associated with the absence of response to neoadjuvant chemotherapy.

PI-14UZ
AVALUZ Study: First Line with Bevacizumab in Combination with Paclitaxel (P) and Gemcitabine (G) in Patients with HER-2 Negative Recurrent or Metastatic BC: PFS Analysis.
Salvador J, Ciruelos E, Codes de Villena M, Jaen A, Gil M, Galan A, Muriás A, Jara C, de la Haba J, Baena JM, Villanueva MJ, Bayo J, Blancas I, Gonzalez E, Perez D, Mel JR, Manso L. Hospital U. de Valme, Sevilla, Spain; Hospital 12 de Octubre, Madrid, Spain; Hospital Virgen Macarena, Sevilla, Spain; Hospital de Jaen, Jaen, Spain; ICO, Bellvitge, Spain; Hospital de Sagunto, Sagunto, Spain; Hospital Insular de Gran Canaria, Gran Canaria, Spain; Fundacion Hospital de Alcorcon, Alcorcon, Spain; Hospital Reina Sofia, Cordoba, Spain; Hospital Puerta del Mar, Cadiz, Spain; Hospital Meixoeiro, Vigo, Spain; Hospital Juan Ramón Jimenez, Huelva, Spain; Hospital San Cecilio, Granada, Spain; Hospital Virgen de las Nieves, Granada, Spain; Hospital Costa del Sol, Marbella, Spain; Hospital Lucas Augusti, Lugo, Spain

Background
The combination of bevacizumab (B) with taxanes, capetcitabine or anthracyclines has shown increased PFS in pts with mBC. The combination with G and P has been evaluated and has demonstrated high response rate and good toxicity profile. The aim of this study is to evaluate the efficacy and toxicity profile of the combination of B with G and P, providing an update of PFS and the toxicity experienced in all patients treated.

Methods
A phase II multicenter, national, open-label study in pts diagnosed of recurrent or metastatic HER-2 negative BC, treated with first line B 10 mg/kg, P 150 mg/m2 and G 2000 mg/m2 day 1 and 15 c/28 d until progression disease, unacceptable toxicity or medical decision. The abstract evaluates efficacy by PFS as primary endpoint and as secondary endpoints: response rate and toxicity profile (NCI CTC v3.0 criteria).

Results
From January 2009 to December 2009, 82 evaluable patients were recruited in 23 sites. The characteristic of the all of patients included (90) are: median age 51.5 (26-81), ER + 68%, PR + 59%, triple negative pts 19%, previous chemotherapy (neoadj or adj) 64.4% (90).

PI-14-04
Prolonged (≥1 Year) Exposure to First-Line Bevacizumab Combined with Paclitaxel in Patients with HER2-Negative Metastatic Breast Cancer Treated in a Routine Oncology Practice Study.
Kuemmel S, Schneegeiss A, Foerster FG, Geberth M, Tesch H, Klare P, Schumacher C, Hollburg W, Soeling U, Schmidt M, Kliniken Essen-Mitte, Essen, Germany; National Center for Tumor Diseases, Heidelberg, Germany; University of Applied Sciences Zwickau, Zwickau, Germany; SPOG-Mannheim, Mannheim, Germany; Oncological Practice Bethanien, Frankfurt, Germany; Oncology Practice Krebsheilkunde für Frauen, Berlin, Germany; St. Elisabeth-Hospital, Köln, Germany; HOPA (Hämatologisch-Onkologische Praxis Altona) im Struenseehaus, Hamburg, Germany; Practice, Kassel, Germany; University Hospital Mainz, Mainz, Germany

Background:
First-line bevacizumab (BEV) combined with weekly paclitaxel (PAC) significantly improves progression-free survival (PFS) and response rate (RR) vs PAC alone in HER2-negative metastatic breast cancer (mBC), as shown in E2100. The benefit of BEV combined with other chemotherapy (CT) agents was demonstrated in AVADO and RIBBON-1. BEV was continued for ≥1 year in 21% of patients in the global ATHENA safety study and in 42% of patients in the JO19901 single-arm Japanese study of BEV–PAC. We analyzed data from the subgroup of patients treated for ≥1 year in a German routine oncology practice study to provide insight into the safety and efficacy of prolonged first-line BEV–PAC.

Methods:
Patients who had received no prior CT for their mBC received BEV–PAC per the European label. Efficacy and safety were documented for up to 1 year (or until progression, death, or BEV discontinuation if earlier) with additional long-term follow-up. Data from patients treated with BEV for ≥1 year were extracted for this analysis.

Results:
By Jan 2011, data were available for 818 patients, of whom 157 (19%) had already received BEV for ≥1 year. Baseline characteristics of this subset relative to the overall population are summarized in the table.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Overall population (n=818)</th>
<th>Subgroup treated with BEV for ≥1 year (n=157)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age, years (range)</td>
<td>58 (26-87)</td>
<td>56 (28-79)</td>
</tr>
<tr>
<td>Age ≥65 years, %</td>
<td>52</td>
<td>59</td>
</tr>
<tr>
<td>Metastatic at diagnosis, %</td>
<td>19</td>
<td>15</td>
</tr>
<tr>
<td>Disease-free interval &lt;12 months, %</td>
<td>24*</td>
<td>14</td>
</tr>
<tr>
<td>≥3 metastatic sites, %</td>
<td>33</td>
<td>32</td>
</tr>
<tr>
<td>TRAIL-negative disease, %</td>
<td>19</td>
<td>13</td>
</tr>
<tr>
<td>Prior endocrine therapy for mBC, %</td>
<td>22</td>
<td>26</td>
</tr>
<tr>
<td>Prior (neo)adjuvant chemotherapy, %</td>
<td>65</td>
<td>62</td>
</tr>
</tbody>
</table>

*109

In 79% of those treated for ≥1 year, BEV was continued as a single agent after discontinuation of CT. The overall RR in patients treated for ≥1 year was 81% (complete response in 20%). Median PFS was 1.7 months (events in 44% of patients) vs 9.4 months in the overall population (events in 68%). Overall survival data are immature, as 81% of those treated for ≥1 year are still alive. The most common grade 3/4 adverse events in patients treated with BEV for ≥1 year were hypertension (11% of patients), pain (10%), and leukopenia (7%). There were no cases of gastrointestinal perforation, arterial
In general, the rate of surgical complications was low. A complication was reported in 11% with EC-T compared to 15.3% reported in EC-TB (p=0.80). Any complication was reported in 3.4% with EC-T and 6.3% with ECB-TB (p=0.11). Necrosis was reported in 3.9% with ECB-TB (p=0.39). Delayed wound healing was reported in 6.7% of the patients treated by EC-T and 7.1% with ECB-TB (p=0.88). The rate of pathological complete response was 19.5% with EC-T compared to 23.2% with ECB-TB (p=0.26). The rate of pathological complete response was 46% in the EC-T arm compared to 71.1% in the ECB-TB arm (p=0.54). The rate of pathological complete response was 46% in the EC-T arm compared to 71.1% in the ECB-TB arm (p=0.54). The rate of pathological complete response was 46% in the EC-T arm compared to 71.1% in the ECB-TB arm (p=0.54).

P1-14-05
Surgical Complications from the GeparQuinto Trial of Patients Receiving Preoperative Bevacizumab.
Universitätsklinikum, Kiel; Praxisklinik, Berlin; Luisenko Krankenhaus, Düsseldorf; Onkologische Gemeinschaftspraxis am Bethanien-Krankenhaus, Frankfurt; Städtisches Klinikum, Karlsruhe; Frauenklinik, Rheinfeld; Universityklinikum, Greifswald; Helios Klinikum Berlin Buch, Berlin; Universitätssklinikum, Freiburg; Henriette Stiftung, Hannover; Universityklinikum, Erlangen; Hämatologisch-Onkologische Schwerpunktpraxis, München; Evangelisches Krankenhaus, Gelsenkirchen; St. Barbara-Klinik, Heessen; Universitätsklinikum, Frankfurt; German Breast Group, Neu-Isenburg

Background: Bevacizumab has been reported to increase the risk of surgical complications, especially in its treated with neoadjuvant chemotherapy.

Methods: In January 2010 it was decided to collect surgical complications prospectively on a specifically developed form. All patients received chemotherapy with epirubicin/cyclophosphamide (EC) followed by docetaxel (T) with or w/o bevacizumab. Patients not responding to the first 4 cycles of EC/T-B were enrolled in another setting. All patients receiving bevacizumab were supposed to be operated at 5 weeks after the last infusion of bevacizumab.

Results: Data from 699 patients have been prospectively collected, 329 received EC-T and 370 ECB-TB. Median age was 48 years in both arms. Median tumor size was 30mm in the ET-T arm vs 35mm in the ECB-TB arm (p=0.13). Multifocal or multicentric disease was present in 21% in the EC-T arm vs 22.4% (p=0.74). 36% of the patients received a sentinel node biopsy prior to start of neoadjuvant chemotherapy. All patients receiving bevacizumab were supposed to be operated at 5 weeks after the last infusion of bevacizumab.

Conclusion: In general, the rate of surgical complications was low. Patients treated with bevacizumab had a numerical increase of surgical complications. However, none of the differences were statistically significant. The intervals from last infusion to surgery as defined by the study protocol appear to be safe.

P1-15-01
Oral Fluoropyrimidine (UFT and S-1) May Augment the Efficacy of Aromatase Inhibitor Via the Down-Regulation of Estrogen Receptor in Estrogen-Responsive Breast Cancer Xenografts.
Nakatsuka M, Saito H, Nakagawa F, Abe M, Uchida J, Shibata J, Matsuo K-I, Noguchi S, Kiniva M, Taito Pharmaceutical Co., Ltd., Tokushima-shi, Tokushima, Japan; Taito Pharmaceutical Co., Ltd., Tsukuba-shi, Ibaragi, Japan; Osaka University Medical School, Osaka, Japan

Breast cancer patients have tended to be more personalized by biomarkers, such as estrogen receptor (ER), HER-2 and other risk factors. Adjuvant endocrine therapy is recommended for all ER-positive breast cancer patients, and the addition of adjuvant chemotherapy to endocrine therapy has been shown to further improve the prognosis of ER-positive breast cancer patients. Addition of an oral fluoropyrimidine, UFT was shown to improve the outcome of patients with luminal A cancer. Based on these studies, the present preclinical study was designed to evaluate a new combination therapy comprised of the aromatase inhibitor anastrozole (ANA) and the oral fluoropyrimidines, UFT and S-1 against luminal A breast cancer cell line MCF-7/Arom 14, which was stably transfected with the cDNA of human aromatase. MCF-7/Arom 14 cells showed a high aromatase activity (111.6±69.6 fmole/mg protein/hr) in vivo. Testosterone failed to induce cell growth of parent MCF-7. But, MCF-7/Arom 14 cells were potentiated by both testosterone and E2 in vitro, and ANA and 5-fluorouracil (5-FU) inhibited cell growth at concentrations of 0.005 to 10 and 0.2 to 5 μM, respectively. The combination of 5-FU and ANA added cell growth inhibition. MCF-7/Arom 14 was implantable in vivo, but it failed to grow in the absence of either testosterone or ANA. Testosterone releasing pellet (Tes-P). The growth of MCF-7/Arom 14 was significantly inhibited by ANA and S-1 or UFT alone vs Control (Tes-P alone) in vivo. The combination of ANA with S-1 or UFT administered using a 21-day consecutive, metronomic-like regimen. On day 22, relative tumor volume (RTV) treated by ANA and UFT or ANA and S-1 was significantly lower than either mono-therapy by Welch’s t-test. Based on RTV change the period required for the RTV to reach 3 was estimated, and its differences between control was designated as growth delay period (GDP). GDP of combination-therapy was 2 to 4 times longer than either mono-therapy.

Antitumor activity against MCF-7/Arom 14

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>RTV on day 28 (mean±SD)</th>
<th>GDP (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>0.67±0.95</td>
<td>9</td>
</tr>
<tr>
<td>ANA alone</td>
<td>3</td>
<td>5.90±0.49</td>
<td>2.4</td>
</tr>
<tr>
<td>UFT alone</td>
<td>20</td>
<td>4.71±0.67</td>
<td>3.0</td>
</tr>
<tr>
<td>UFT+ANA</td>
<td>20+3</td>
<td>3.83±0.51**</td>
<td>4.3</td>
</tr>
<tr>
<td>S-1 alone</td>
<td>10</td>
<td>3.90±0.62</td>
<td>5.5</td>
</tr>
<tr>
<td>UFT-ANA</td>
<td>10+3</td>
<td>2.45±0.59*</td>
<td>9.5</td>
</tr>
</tbody>
</table>

*p<0.05 and 0.01, respectively (intersection-union test), n=10.

To investigate the mechanisms by which fluoropyrimidines enhance the antitumor activity of ANA, the protein and mRNA expression levels of ER-α in tumor tissue after treatment with S-1, ANA, and the typical chemotherapeutic agents doxorubicin (ADM) or paclitaxel (TXL) were analyzed by immuno-histostain and RT-PCR, respectively. The protein and mRNA expression of ER-α were markedly decreased by S-1 or S-1+ANA, but not for ADM or TXL. The reduced ER-α level might increase antitumor activity of ANA in addition to the decreased estrogen production. As activity of dihydropyrimidine dehydrogenase (DPD) in breast cancer is
higher, UFT or S-1 which is resistant against DPD would be suitable compared other 5-FU derivatives. Therefore, the combination of ANA and S-1 might yield a greater benefit than other chemotherapeutic agents in postmenopausal women with Luminal A breast cancer.

P1-15-02

Nanoparticles Overcome the Decreased Responsiveness of Breast Cancer Cells to a Chemotherapeutic Drug in the Presence of Adipocytes.

DeAngel RE, Sandoval MA, Lansakara-P DSS, Dunlap SM, Hursting SD, Cui Z. The University of Texas at Austin, Austin, TX; UT-MD Anderson Cancer Center, Smithville, TX

Background

Excess adipose tissue plays a role in increasing breast cancer risk and tumor aggressiveness. However, the role of mature adipocytes in tumor progression and response to therapy remains unclear. Despite advances in breast cancer treatment, obese patients still show decreased response to chemotherapy. The incorporation of nanoparticles (NPs) in breast cancer therapeutics represents a novel approach for improving therapy efficacy. NPs increase the drug accumulation in the tumor site resulting in increased drug disposition, decreased cytotoxicity and decreased adverse side effects. However, few studies have evaluated the response to chemotherapy-loaded-NPs in the context of obesity. Gemcitabine is a widely used nucleoside analog that inhibits DNA synthesis. Previously, we prepared a novel gemcitabine formulation by incorporating 4-(N)-stearoyl gemcitabine in solid-lipid NPs (Gem-NPs). We hypothesized that the enhancement of tumor growth and decreased response to therapy in the presence of adipocytes can be offset by treatment with Gem-NPs. To test this we used an in vitro model of adipocyte-conditioned media (CM) and investigated the effects of mature adipocytes on breast cancer cells.

Methods

Murine 3T3-L1 adipocytes were induced to differentiate; adipocyte-CM from these cells was collected, centrifuged and used for most assays. Murine (M-Wnt, E-Wnt) and human (MCF-7) cancer cells were grown in RPMI and DMEM, respectively, supplemented with 10% FBS and 1% penicillin/streptomycin at 37°C with 5% CO2. Once the cells were confluent, cells were split and then cultured with a matrigel invasion assay. CM from undifferentiated 3T3-L1 adipocytes (fibroblasts-CM) were used as a negative control.

Results

After 48 h of culture with CM, all cells (M-Wnt, E-Wnt, and MCF-7) showed increased proliferation (p < 0.05) and the invasiveness capacity was increased in the presence of adipocyte-CM after 20 h in culture. Cells cultured with adipocyte-CM were less responsive to treatment with gemcitabine (p < 0.01) relative to cells cultured with fibroblasts-CM. The apoptotic response to gemcitabine was decreased, and s-phase arrest was not observed in the presence of adipocyte-CM. However, treatment with Gem-NP overcame the decrease in gemcitabine responsiveness caused by adipocyte-CM, restoring apoptotic response and S-phase arrest.

Conclusion

Our results strongly support the role of mature adipocytes in promoting tumor growth and invasiveness while decreasing the response to a standard chemotherapy. Our findings also suggest that incorporating a chemotherapeutic drug into NPs can potentially decrease chemotherapy resistance, particularly in obese patients. Animal studies are underway to evaluate this response in an in vivo mouse model of obesity.

P1-15-03

Multistage Vectored Nanotherapeutics for Breast Cancer Metastasis.

Shen H. The Methodist Hospital Research Institute, Houston, TX

Significant progresses have been made on overall survival rates in breast cancer. The five-year survival rate of women diagnosed with Stage I-III breast cancer has been steadily improving decades over decades. These improvements in survival have primarily been attributed to availability of modern diagnostic tools that allow for early detection, and the increased use of adjuvant systemic therapies. However, the situation with breast cancer metastasis is discouraging, with only marginal improvement in some reports to no improvement at all in other studies in survival of the relapsed metastatic patients. More breast cancer patients are killed by cancer metastasis than by the primary cancer.

Nanotechnology has played an important role in the fight against breast cancer in the last decade. The first nano-liposomal formulation of doxorubicin (Dox), Doxil, was FDA approved in 1994, and is currently used to treat multiple cancer types including recurrent and refractory breast cancers. More nano-formulated drugs have since been approved. These nanotherapeutics work based on the enhanced permeability and retention (EPR) effect, and still cause severe toxicity to the body, however. To overcome these defects of the first generation of nanotherapeutics, we have developed a silicon-based multistage vector (MSV) system with the aim to specifically delivery drugs to cancer tissues with the least toxicity to the normal organs. These are particle-in-particle “Russian Doll” systems with each stage designed to provide transport across a set of sequential biological barriers, and provide associated levels of targeting specificity. We have demonstrated highly reproducible therapeutic efficacy with this system to deliver the conventional chemotherapy drugs and siRNA oligos.

We have generated mouse lung metastasis models with the human breast cancer cell line MDA-MB-231 which was engineered with a luciferase gene, and used multiple doxorubicin formulations to treat the tumor mice. These included free doxorubicin (3 mg/kg weekly), Doxil (6 mg/kg biweekly), a new formulation of doxorubicin in micelles (miDox, 6 mg/kg biweekly), and miDox in MSV (MSV/ Dox, 6 mg/kg biweekly). Tumor growth in the lung was monitored by bioluminescence with a Xenogen IVIS200 optical in vivo imaging system. As expected, treatment with free Dox caused significant weight loss indicating drug toxicity, most likely to the heart. Tumor growth in the free Dox treatment group halted initially, but resumed two weeks later. The tumor cells in these animals might have developed resistance to Dox during the treatment. Doxil treatment also triggered weight loss initially. This correlates with the cardiac side effect of the drug observed in clinic. Neither miDox or MSV/ Dox caused weight loss. Tumor growth was also inhibited in mice treated with both formulations. The result was much more significant with MSV/Dox than with miDox. No tumor cells were observed in the lung after a 6-week treatment with MSV/Dox, while 40% of the miDox-treated mice still had residue, but detectable tumor cells in the lung. These results indicate that we have developed a powerful system for tumor-specific delivery of therapeutic in the treatment of breast cancer metastasis.
**P1-16-01**  

Masuda N, Izawa H, Ohno S, Rai Y, Sato Y, Ohsumi S, Hashigaki S, Nishizawa Y, Saeki T, Noguchi S. NHO Osaka National Hospital, Osaka, Japan; Aichi Cancer Center Hospital, Aichi, Japan; Kyushu Cancer Center, Fukuoka, Japan; Sagarara Hospital, Kagoshima, Japan; Nagoya Medical Center, Aichi, Japan; Shikoku Cancer Center, Ehime, Japan; Pfizer Japan Inc., Japan; Saitama Medical University International Medical Center, Saitama, Japan; Osaka University, Osaka, Japan

**Background**  
The steroidal irreversible aromatase inhibitor (AI) exemestane (E), the non-steroidal reversible AI anastrozole (A) and tamoxifen (T) are approved for the first-line treatment of the postmenopausal women with hormone receptor (HR) positive advanced breast cancer (ABC) in Japan. Although there are some studies which compare the efficacy and safety of AIs and T in the first-line disease setting, the number of studies that compare efficacy and safety of AIs is limited. We conducted this multicenter, randomized, double-blinded non-inferiority study, to evaluate the time to progression (TTP) in HR positive ABC randomized to therapy with E or A.

**Methods**  
Patients (pts) who were ≥20 years [yrs], postmenopausal, ECOG PS ≤1 and had HR positive ABC that recurred after the adjuvant therapy or metastatic disease settings were eligible and randomized (1:1) to 25 mg/day of E or to 1 mg/day of A. Data were evaluated for non-inferiority of E compared to A defined as the upper limit of a two-sided 95% confidence interval (CI) of the hazard ratio (HR) of TTP being less than or equal to 1.25. The primary endpoint was TTP assessed by the independent radiological images review committee (RIRC). Secondary endpoints included TTP by investigator, time to treatment failure, overall survival (OS), objective response rate (ORR), clinical benefit rate, and safety.

**Results**  
A total of 298 pts from 58 sites were randomized to E (n=149; mean age: 63.4 yrs) or A (n=149; mean age: 64.0 yrs). The mean BMI for the E and A arms were 23.0 kg/m² and 23.6 kg/m², respectively. Six pts (2 pts in E arm, 4 pts in A arm) were excluded from the full analysis set due to lack of evaluation for anti-tumor response after study medication started. Median TTP (as per RIRC) was 13.8 months (M) vs 11.1 M for E vs. A, respectively (HR 1.007; 95% CI: 0.771–1.317). Median TTP (Investigator) was 13.8 M vs. 13.7 M for E vs. A, respectively (HR 1.059; 95% CI: 0.816–1.374). The median OS for A treated pts was 60.1 M, OS for E was not reached (as of data cut-off: December 8, 2010). ORR for E was 43.9% (95% CI: 35.3–52.8) and 39.1% (95% CI: 30.6–48.1) for A. Other analyses, including sub-population analyses are ongoing.

The incidence of treatment related adverse events (AEs) in E arm was 71.1% (n=106) and in A arm 59.7% (n=89); the AEs were mostly grade 1 and 2 in 61.7% (n=92) and 53.7% (n=80) of pts respectively. They were expected and manageable. Treatment related SAEs were similar in both groups: 6 (4.0%) in E arm and 5 (3.4%) in A arm. The most common AEs for E were hot flushes (22.1%), arthralgias (16.8%), musculoskeletal stiffness (11.4%) and γ-GTP increased (10.1%); in A arm, hot flushes (14.8%) and arthralgia (16.8%) were observed in >10% pts.

**Conclusions**  
Although median TTP (RIRC) of E is slightly improved compared with that of A, the result of TTP did not meet the non-inferiority criteria. There were no significant differences found between E and A in ORR. Although AEs in E were numerically higher, the observed AE profiles were similar to those previously reported for E and A. This study shows that E is comparable to A in efficacy and safety.

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**P1-17-01**  

Ryan PD, Neven P, Blackwell KL, Dirix LY, Barrios CH, Miller, Jr WH, Fein LE, Fenton D, Benner RJ, Meech SJ, Paccagnella L, Sleight B, Yee D, Goss PE. Fox Chase Cancer Center, Philadelphia, PA; Universitair Ziekenhuis Leuven, Leuven, Belgium; Duke University Medical Centre, Durham, NC; Sint-Augustinus, Oncologisch Centrum, Antwerp, Belgium; PUCRS School of Medicine, Porto Alegre, Brazil; Lady Davis Institute for Medical Research, Jewish General Hospital, Segal Cancer Center, McGill University, Montreal, Canada; Centro Oncologico Rosario, Santa Fe, Argentina; Cross Cancer Institute, Edmonton, Canada; Pfizer Inc., Groton, CT; University of Minnesota, Minneapolis, MN; Massachusetts General Hospital Cancer Center, Boston, MA

**Background**  
Figitumumab (CP-751,871) is a fully human IgG2 monoclonal antibody that inhibits IGF-1R. Exemestane profoundly reduces estrogen production and has activity as first-line treatment in postmenopausal metastatic hormone receptor-positive (HR+) breast cancer (BC). Preclinical studies suggest cross-talk between the IGF-1R and estrogen receptor pathways. Combining agents that inhibit these pathways could benefit women with advanced BC.

**Methods**  
This was an open-label, multicenter, randomized, phase II trial of first-line figitumumab + exemestane (Arm A) vs. exemestane alone (Arm B) in HR+ postmenopausal advanced BC. Eligibility included: age >18 yrs with Stage IIB or IV disease; baseline ECOG PS ≤2. A high rate of grade [G] 3 and 4 hyperglycemia after figitumumab treatment prompted a protocol amendment in February 2010 requiring patients (pts) to have a baseline HbA1c <5.7%. Patients received exemestane 25 mg po daily ± intravenous figitumab 20 mg/kg every 21 days. The primary endpoint was progression-free survival (PFS) in pts with baseline HbA1c <5.7%. Secondary endpoints included clinical benefit rate (CBR) and safety.

**Results**  
Between 2007 and February 2011, 103 pts were randomized to Arm A and 102 to Arm B. Of these, 37 pts in Arm A and 40 pts in Arm B had baseline HbA1c <5.7%. These data are from an unplanned interim analysis which preceded premature study termination by the Sponsor for clinical development reasons and to expedite availability of data collected to date. Baseline demographics were balanced between arms. PFS, CBR and safety are summarized in Table 1.
**Table 1**

<table>
<thead>
<tr>
<th>Table 1</th>
<th>HbaA1c &lt;5.7%</th>
<th>Arm A</th>
<th>Arm B</th>
<th>Arm A</th>
<th>Arm B</th>
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<tbody>
<tr>
<td>Median PFS, months</td>
<td>11.3</td>
<td>9.1</td>
<td>10.3</td>
<td>9.1</td>
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</tr>
<tr>
<td>(Events)</td>
<td>(18)</td>
<td>(28)</td>
<td>(106)</td>
<td>(78)</td>
<td></td>
</tr>
<tr>
<td>HbA1c 3+3</td>
<td>0.63</td>
<td>1.096</td>
<td>0.624</td>
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</tbody>
</table>

**Table 2**

<table>
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<tr>
<th>Table 2</th>
<th>Hyperglycemia 24/0/0</th>
<th>Hyperglycemia 25/3/3</th>
<th>Hyperglycemia 33/7/4</th>
<th>Hyperglycemia 20/2/3</th>
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<tr>
<td>Arm A</td>
<td>24</td>
<td>10</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Arm B</td>
<td>22</td>
<td>3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Arm A HbaA1c &lt;5.7%</td>
<td>22</td>
<td>3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Arm B (t=10)</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Arm B (t=30)</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

**Conclusions:**

The addition of figitumumab to exemestane was tolerable but overall did not improve PFS compared with exemestane alone in pts eligible for this trial. However, a trend toward benefit with figitumumab combined with exemestane in pts without evidence of baseline metabolic syndrome (HbaA1c <5.7%) was observed together with improved tolerability; further study with this combination may be warranted.

**P1-17-02**

A Phase 1/2 Study of SAR245408 (S08) in Combination with Trastuzumab (T) or Paclitaxel (P) and T in Patients with HER2+ Metastatic Breast Cancer (MBC) Who Progressed on a Previous T-Targeted Regimen.

**Methods:**

We are conducting a phase I study to determine the maximum tolerated dose (MTD), pharmacokinetic (PK) and pharmacodynamic profiles (PD), and preliminary anti-tumor activity of veliparib in combination with CP and V in patients (pts) with pretreated metastatic breast cancer.

**DISCUSSION:**

S08 can be combined with either T or with T+P. Additional safety, PK and efficacy data will be presented from phase 1.

**P1-17-03**

Withdrawn by Author

**P1-17-04**

Phase 1 Study of PARP Inhibitor ABT-888 (Veliparib) in Combination with Cisplatin and Vinorelbine for Patients with Advanced Triple Negative Breast Cancer and/or BRCA4-Mutation Associated Breast Cancer.

Rodler ET, Specht JM, Gadi VK, Kurland BF, Griffin MJ, Hammond JJ, Gralow JR, University of Washington/Seattle Cancer Care Alliance, Seattle, WA; Fred Hutchinson Cancer Research Center, Seattle, WA

**Background:**

Inhibitors of poly(ADP-ribose) polymerase (PARP) have shown preclinical and clinical activity in targeting tumors with pre-existing DNA repair defects, in particular BRCA1 and BRCA2 deficient tumors. Cisplatin (CP) has demonstrated synergy with ABT-888 (veliparib) in breast cancer xenograft models and has anti-tumor activity in triple negative (TN) and BRCA4-1 deficient breast cancer. Vinorelbine (V) combined with CP has shown safety and efficacy in patients with pretreated metastatic breast cancer. Methods: We are conducting a phase I study to determine the maximum tolerated dose (MTD), pharmacokinetic (PK) and pharmacodynamic profiles (PD), and preliminary anti-tumor activity of veliparib in combination with CP and V in patients (pts) with metastatic breast cancer who are either TN or who have BRCA1 or BRCA2 mutation associated breast cancer. Cohorts receive escalating doses of ABT-888 orally BID days 1-14, CP 75 mg/m2 intravenously (IV) day 1 and V 25 mg/m2 IV days 1,8 every 21 days, in a 3+3 design. Results: As
of 6/14/11, 18 eligible female pts have been enrolled. The median age at registration was 50 years (range 34-78 years). Sixteen pts received at least one prior metastatic regimen (range 0-8). Three pts had previously treated brain metastases. **BRCA** mutation status was as follows: **BRCA1**+ (3 pts); **BRCA2**+ (2 pts); confirmed mutation negative (8 pts); unknown (5 pts). Four pts received the 20 mg BID veliparib dose (one patient in the cohort died of progressive disease early in cycle 1 and was replaced), 3 received 30 mg BID, 6 received 40 mg BID, and 5 have been enrolled at dose level 4 (60 mg BID). Dose limiting toxicities occurred in one patient at the 40 mg BID dose (grade 4 thrombocytopenia), and one patient at the 60 mg BID dose (grade 3 neutropenic fever). Adverse events are typical for a platinum-based chemotherapy regimen and include nausea, fatigue, thrombocytopenia, and neutropenia. MTD has not been reached. Of 11 pts evaluable for radiographic response to date, 6 (55%) had a PR (3 of whom have a **BRCA** mutation) and 5 (45%) had stable disease. Correlative studies will use immunohistochemistry and gene expression array to evaluate the profile of TN breast cancer and predictors of response to treatment. **Conclusion:** Veliparib in combination with CP and V has been generally well tolerated to date. Objective anti-tumor activity was seen in **BRCA** mutation carriers and in pts with sporadic TN breast cancer. PK, PD, and biomarker analysis is underway. Enrollment continues in the dose escalation cohorts.

**P1-17-05**

**Preliminary Results of a Randomized Phase 2 Study of PD 0332991, a Cyclin-Dependent Kinase (CDK) 4/6 Inhibitor, in Combination with Letrozole for First-Line Treatment of Patients (pts) with Post-Menopausal, ER+, HER2-Negative (HER2–) Advanced Breast Cancer.**

Finn RS, Crown JP, Boer K, Lang I, Parikh RJ, Patel R, Schmidt M, Hagenstad C, Lim H, Pinter T, Amadori D, Chan D, Dicelmman RA, Walshe J, Brezuzna A, Kim ST, Randolph S, Slamon DJ. University of California at Los Angeles, Los Angeles, CA; Irish Cooperative Oncology Research Group, Dublin, Ireland; Szent Margit Koriha, Budapest, Hungary; National Institute of Oncology, Budapest, Hungary; Comprehensive Cancer Centers of Nebraska, Henderson, NV; Comprehensive Blood and Cancer Center, Bakersfield, CA; University Hospital Mainz, Mainz, Germany; Suburban Hematology-Oncology Associates, Lawrenceville, GA; British Columbia Cancer Agency, Vancouver, BC, Canada; Peto Aladar Megyei Okato Korhau, Gyor, Hungary; Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori, Meldola, Italy; Cancer Care Associates Medical Group, Redondo Beach, CA; Central Coast Medical Oncology Corporation, Santa Maria, CA; Pfizer Oncology, La Jolla, CA; Pfizer Oncology, New York, NY

**Background:** PD 0332991 is an orally bioavailable selective inhibitor of CDK 4/6 and prevents cellular DNA synthesis by inhibiting progression of the cell cycle from G1 into the S phase. Preclinical evaluations suggest that reduction in **CDK2A** (p16) expression and cyclin D1 (CCND1) overexpression confer susceptibility to PD 0332991 (Finn 2009). In addition, PD 0332991 was synergistic in combination with tamoxifen in vitro in ER+ human breast cancer cell lines. Based on these observations, a phase 1/2 study in combination with letrozole as first-line therapy for advanced ER+ post-menopausal breast cancer was initiated. The phase 1 part of the study (completed) determined the recommended phase 2 dose to be PD 0332991 125 mg QD on Schedule 3/1 (3 weeks on treatment followed by 1-week off treatment) in combination with letrozole 2.5 mg QD. The combination was generally well tolerated and encouraging antitumor activity was observed. We present preliminary data from the randomized Phase 2 portion comparing letrozole alone to letrozole plus PD 0332991.

**Methods:** The Phase 2 portion of the study is designed as a two-part study; we present data from Part 1. In both parts, eligible patients are randomized 1:1 to letrozole 2.5 mg QD alone (control) or PD 0332991 125 mg QD on schedule 3/1 and letrozole 2.5 mg QD (treatment, tx). Part 1 enrolled post-menopausal women with ER+, HER2– cancer using only ER+, HER2– as a selection criteria. Part 2 is now enrolling post-menopausal women with ER+, HER2– breast cancer with CCND1 amplification and/or loss of p16 by FISH (target N=150). The primary endpoint is progression-free survival (PFS); secondary endpoints include overall survival, response rate, safety, and correlative studies. Pts are stratified for disease site and length from prior adjuvant therapy. Pts continue assigned study treatment until disease progression, unacceptable toxicity, or consent withdrawal and are followed every 2 months to assess disease status. Tumor tissue was required for participation. **Results:** 66 patients have been randomized in Part 1. At the time of data cut-off (April 2011) median duration of treatment is 20 (range 4–64) wks for control and 27 (2-59) wks for tx. Dose reductions occurred in 9 pts on the tx arm and none on the control arm. There are no complete responses. The number of partial responses for pts with measurable disease are similar between arms (4/22 control vs 5/24 in tx). The number of pts with stable disease≥ 24 weeks was higher in the tx arm (5 vs 8). The number of pts with best response of progressive disease is lower in the treatment arm (2 vs 6). PFS data are immature. Twelve pts remain on control vs. 21 on tx. As in the Phase I portion of the study, the most common treatment-related AEs were neutropenia and leukopenia without febrile neutropenia. Biomarker studies for CCND1 amplification, p16 loss, RB status, and Ki67 are ongoing. **Conclusion:** The combination of PD 0332991 and letrozole is well tolerated as first-line treatment of ER+, HER2– post-menopausal breast cancer. Updated efficacy data and biomarker data will be presented.

**P1-17-06**

**A Phase II Trial of the CDK 4/6 Inhibitor PD0332991 in Women with Advanced Breast Cancer.**


**Background:** Dysregulation of the G1/S checkpoint of the cell cycle is a feature of many breast cancers. PD0332991, a potent oral inhibitor of cyclin-dependent kinases (CDKs) 4 and 6 is well-tolerated and has demonstrated activity in a phase I trial in a variety of solid tumors. The combination therapy (tamoxifen plus PD0332991) was generally well tolerated with a manageable safety profile. In the phase II dose of 125 mg daily on a 3-week on/1-week off schedule. Preclinical data suggest that this agent is most active in ER+ (luminal) breast cancers. We are performing a phase II study of PD0332991 in women with advanced breast cancer, one of several parallel disease cohorts under study.

**Methods:** Patients with histologically-confirmed stage IV breast cancer were eligible if they had primary or metastatic tissue from prior adjuvant therapy. Pts are stratified for disease site and length from prior adjuvant therapy. Pts continue assigned study treatment until disease progression, unacceptable toxicity, or consent withdrawal and are followed every 2 months to assess disease status. Tumor tissue was required for participation. **Results:** 66 patients have been randomized in Part 1. At the time of data cut-off (April 2011) median duration of treatment is 20 (range 4–64) wks for control and 27 (2-59) wks for tx. Dose reductions occurred in 9 pts on the tx arm and none on the control arm. There are no complete responses. The number of partial responses for pts with measurable disease are similar between arms (4/22 control vs 5/24 in tx). The number of pts with stable disease≥ 24 weeks was higher in the tx arm (5 vs 8). The number of pts with best response of progressive disease is lower in the treatment arm (2 vs 6). PFS data are immature. Twelve pts remain on control vs. 21 on tx. As in the Phase I portion of the study, the most common treatment-related AEs were neutropenia and leukopenia without febrile neutropenia. Biomarker studies for CCND1 amplification, p16 loss, RB status, and Ki67 are ongoing. **Conclusion:** The combination of PD 0332991 and letrozole is well tolerated as first-line treatment of ER+, HER2– breast cancer. Updated efficacy data and biomarker data will be presented.

**241s** Cancer Res; 71(24 Suppl.) December 15, 2011
Results: 36 patients were screened, 32 (89%) stained positive for Rb, and 14 have enrolled on study. The only reported toxicities are neutropenia (7 patients, 4 grade 3/4), thrombocytopenia (1 patient, grade 1) and fatigue (1 patient, grade 2). 3 patients (23%) have had dose interruptions and 5 (38%) have had dose-reduction for neutropenia, though no episodes of febrile neutropenia have occurred. Among 11 patients assessable for response to date, there is 1 (7%) partial response (PR), 6 (43%) with stable disease (SD) and 4 (29%) with progressive disease (PD). 3 of 6 patients with stable disease have received greater than 6 months of therapy, and these sustained responses have occurred with dosing as low as 50 mg/day. All PR/SD have occurred in patients with ER+ tumors; all PD have been in patients with triple negative (ER-/PR-/Her2-) disease. The cyclin D1 status of all patients are being assessed. Of the 10 ER+ patients, 3 are cyclin D1 amplified, 5 are non-amplified and 2 are pending assessment. 2 in 4 cyclin D1 non-amplified patients had SD, while 2 of 2 evaluable patients with amplification had PD. PK and PD analyses are in progress.

Conclusions: PD 0332991 is an extremely well-tolerated, oral CDK 4/6 inhibitor that demonstrates prolonged single-agent activity in ER+ breast cancer patients who have progressed on hormonal therapy. These data have prompted expansion of this breast cancer cohort to further delineate activity and translational studies examining predictors of response are underway.

P1-17-07
Phase II Trial of RAD001 Plus Carboplatin in Patients with Triple-Negative Metastatic Breast Cancer.

Background: RAD001 is an oral mTOR inhibitor that has exhibited activity in breast cancer. Triple negative breast cancer patients are unable to repair double stranded DNA breaks and hence have sensitivity to platinum agents that cause interstrand cross-links. Rapamycin acts synergistically with platinum agents to induce apoptosis and to repair double stranded DNA breaks and hence have sensitivity to platinum agents that cause interstrand cross-links. Consequently, we are investigating a phase II trial of RAD001 plus carboplatin in patients with triple negative metastatic breast cancer.

Methods: This study is a phase II trial that will enroll 25 women with hormone-refractory, HER2-negative breast cancer which has progressed to first or second line therapy. Eligibility criteria include hormone receptor positive, triple-negative breast cancer with measurable disease, age 18-70, Karnofsky performance status of 70-100%, adequate marrow, prior trastuzumab is not allowed. Patients will be treated with 10mg of RAD001 orally daily for 21 days, followed by 1 week of rest. Carboplatin AUC=6 will be administered intravenously every 3 weeks.

Results: Fourteen patients of a planned 25 have been recruited thus far. Median age is 58.5. Median number of prior regimens is 2 (0-3). Of the 7 patients assessable for response at this time, there have been 2 PR’s and 5 patients with SD. One SD was achieved in a patient progressing on single agent carboplatin at study entry. Median duration of SD + PR is 28.5 weeks (5 patients have ongoing response ranging from 8-46.5 weeks). Five of 8 patients assessable for toxicity had grade 3 or 4 thrombocytopenia and 2 patients had grade 3 neutropenia. No cases of febrile neutropenia were observed. Four patients have required blood transfusion and one patient has required platelet transfusion. All patients have had treatment held and/or dose reductions secondary to hematological toxicity, however, since amended carboplatin dose the regimen has been very well tolerated with only one out of six patients) with grade 3 neutropenia and grade 3 thrombocytopenia. There have been no non-hematological grade 3 or 4 toxicities. Conclusions: Clinical benefit was observed in all 7 assessable patients. Dose limiting thrombocytopenia was an unexpected side effect requiring protocol amendment. We continue to accrue study subjects at the amended dosing.
3 AEs were limited to 2 pts: 1 with an asymptomatic and reversible elevation in serum amylase, and the other with abdominal pain and diarrhea requiring a dose reduction to 175 mg/m². Of the 10 pts evaluable for efficacy, there is 1 confirmed partial response (PR), 1 minor response (MR) and 2 pts with stable disease (14%). The pt with MR had TNBC and was taken off-study due to a complicated pneumonia requiring hospitalization (unrelated). The remaining 3 pts had HER2+/BC; 2 of these 3 (1 PR, 1 SD) are continuing on study and have completed 4 cycles thus far. Updated toxicity and efficacy data will be presented.

Conclusions: These data show activity for single agent ganetespib in different subtypes of breast cancer. Ganetespib was well tolerated, with expected gastrointestinal toxicity that was mild in nature and manageable in all patients. Accrual continues and we will present updated data at the meeting.

P1-17-09
A Phase 1/2 Dose-Escalation Study of SAR245408 (S08) or SAR245409 (S09) in Combination with Letrozole (L) in Subjects with Hormone Receptor-Positive and HER2-Negative (HR+/HER2-) Breast Cancer (BC) Refractory to a Nonsteroidal Aromatase Inhibitor (AI).

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Background: Upregulation of PI3K activity is a common molecular mechanism involved in resistance to AIs. S08 is a potent, orally bioavailable, pan-PI3K inhibitor. S09 is a potent, orally bioavailable inhibitor of PI3K which also possesses mTOR inhibitory activity. Both compounds exhibit robust PI3K and ERK pathway inhibition in paired human tumor biopsy samples from phase 1 studies (Edelman G, et al. ASCO 2010; Brana I, et al. ASCO 2010).

Methods: This ongoing, open-label, multicenter, phase 1/2 study (NCT01082068) was designed to evaluate the safety and tolerability of L in combination with either S08 (Arm 1) or S09 (Arm 2). Eligible female patients (pts) were ≥18 yrs, ECOG PS 0-1, with advanced or metastatic BC refractory to an AI. Up to 4 prior treatment regimens (hormonal excluded) for MBC were allowed. Tumor response was assessed every 6 wks per mRECIST 1.0. Treatment beyond wk 12 was based on response: pts with partial response (PR) continued with open-label cabozantinib, those with stable disease (SD) were randomized to cabozantinib or placebo, and those with progressive disease were discontinued. The primary endpoint was objective response rate (ORR) in the lead-in period, and progression-free survival in the randomized period. Post-hoc evaluations of bone scans were conducted.

Results: 20 MBC pts were enrolled with a median age of 55 years (range: 31-71). 19 pts had invasive ductal carcinoma (1 pt with inflammatory MBC), and 1 pt had invasive lobular carcinoma. 75% of pts were ER/PR [+]; 15% were HER2/neu [+]. 85% of pts had visceral disease including 65% with liver metastases; 70% had bone disease. The median number of prior regimens was 3; 45% received prior anti-VEGF therapy. Most common related adverse events ≥ Grade 3 were fatigue/asthenia, 20%; and hand-foot syndrome, 20%. Dose reductions and permanent discontinuations for AEs occurred in 35% and 15% of pts, respectively. Soft tissue effects: At wk 12, ORR
per RECIST was 10% and overall disease control rate (PR+SD) was 45%. Notably, tumor shrinkage was observed in 15/16 pts (94%) with ≥1 post-baseline tumor assessment. Bone effects: 2/3 pts evaluable by bone scan at baseline had partial resolution of lesions and one pt had SD on follow-up bone scans as early as Wk 6. Out of 7 pts receiving narcotics for bone pain, 3 pts reported improved pain and 2 pts had decreased narcotic use, per investigator. The majority of pts analyzed had declines in plasma CTx, an osteoclast marker. Additional enrollment and further bone marker analyses are ongoing.

Conclusions: Cabozantinib exhibits clinical activity in pts with MBC regardless of receptor and prior treatment status as reflected by high rate of tumor regression and effects on bone metastases. Cabozantinib is generally well tolerated and the safety profile is comparable to that seen with other tyrosine kinase inhibitors.

P1-18-01
Z-ACT1: Zometa Combined with Standard Therapy in Patients with Metastatic Breast Cancer Further Decreases the Proportion of Patients with CTC Counts of 5 or above.
Bruzicky A, Beck J, Dakhil S, Hallmeyer S, Tezcan H, Yardley D, Tran D, Warsi G, Culver K. McGee Women’s Hospital of University of Pittsburgh Medical Center, Pittsburgh, PA; Highlands Oncology Group, Fayetteville, AR; Cancer Center of Kansas, Wichita, KS; Oncology Specialist, SC; Park Ridge, IL; Kootenai Cancer Center, Post Falls, ID; Sarah Cannon Research Institute, Nashville, TN; Tennessee Oncology, PLLC, Nashville, TN; Novartis Pharmaceuticals Corporation, East Hanover, NJ

Introduction: Zoledronic acid (ZOL) has been shown to reduce the risk of recurrence and residual tumor size in the adjuvant/neoadjuvant setting in patients with early/intermediate-stage breast cancer (BC). In addition, ZOL combined with neoadjuvant chemotherapy reduced numbers of disseminated-tumor cells in the bone marrow compared with chemotherapy alone. However, the activity of ZOL on disseminated- or circulating-tumor cells (CTCs) in patients with metastatic breast cancer (MBC) is not well defined. Cristofanilli M, et al (J Clin Oncol. 2005; 23: 1420-30) reported that CTCs in MBC are an independent predictor of overall survival (OS) and progression-free survival (PFS). Accordingly, this study is evaluating the potential anticaner benefit of adding ZOL to standard therapy in patients with newly diagnosed MBC as assessed by the change in CTC count from baseline to 3-5 weeks.

Methods: Eligible patients had HER2-negative MBC, newly diagnosed or at first relapse after adjuvant therapy with or without bone metastases. In this open-label 3-arm study, patients without bone metastases were randomized to standard therapy + ZOL every 3-4 weeks for the first 6 months (Arm A) or standard therapy + ZOL during month 6-12 after standard therapy initiation (Arm B). All patients with bone metastases received ZOL every 3-4 weeks (Arm C). The primary endpoint is PFS. Secondary endpoints include the proportion of patients with CTCs ≥5 or <5 per 7.5 mL of peripheral blood 3-5 weeks after standard therapy initiation. Data were compared with historical controls (patients with MBC receiving first-line standard treatment alone; Cristofanilli M, et al. 2005). CTCs were quantified using CellSearch™.

Results: In Z-ACT1, 29 previously untreated MBC patients with bone metastases were enrolled in Arm C, all of whom had ≥1 CTC at study entry (range, 1-117). 53% received hormonal therapy alone, 42% chemotherapy alone, and 5% received various combinations. In patients receiving standard therapy + ZOL, the percentage of patients with CTC ≥5 decreased from 55% to 25% at 3-5 weeks. At baseline, the median uNTX level was 46.5 (n = 10) in patients with < 5 CTCs and 57 in patients with ≥5 CTC (n = 13). At 3-5 weeks, the median decrease from baseline in uNTX was 74% (n = 10) in the < 5 CTC group and 25% (n = 5) in the ≥5 CTC group. At 3-5 weeks, the median uNTX levels in the <5 and ≥5 CTC groups were 12 and 22 nmol bce/mmol, respectively. This study has now been modified to a 2-arm study (standard therapy +/- ZOL) in MBC patients with no bone metastases. CTC, uNTX, and PFS data will be presented from this new head-to-head analysis. Changes in CTCs out to 6 months and correlation with uNTX and PFS in this original bone metastasis cohort will also be presented.

Conclusions: This preliminary analysis suggests that the addition of ZOL to standard therapy in women with bone metastases from MBC results in a further decrease in CTC numbers at 3-5 weeks after initiation of therapy.

P1-18-02
Descriptive Analysis of the Management of Breast Cancer Patients with a Solitary Lesion Diagnosed with 18-Fluorodeoxyglucose Positron Emission Tomography/Computed Tomography Scan.
Redmana S, De Carolis V, Racca M, Zago G, Varetto T, Montemurro F, Aglietta M, Evangelista L. Fondazione Piemontese per l’Oncologia - IRCC, Candiaio, TO, Italy; Istituto Oncologico Veneto - IOV, Padova, PD, Italy

Background: A small percentage of breast cancer (BC) patients (pts) will develop oligo-metastatic disease (OMD), often with a solitary lesion (SL). Growing evidences suggest a survival benefit for pts who undergo local treatment for SL. However, 18-fluorodeoxyglucose (FDG) positron emission tomography/computed tomography (PET/CT) scan might improve the detection of truly SL, an important prerequisite for local treatments. We describe pattern of presentation and management of pts with SL diagnosed by FDG-PET/CT.

Methods and Patients: From a bi-institutional database we retrospectively identified 137 pts who underwent a PET/CT because of rising tumor markers (95, 69%) or clinical/radiological suspect of disease recurrence (42, 31%). Median time from surgery of primary tumor to metastatic progression was 48 months (range 6-216 months). Comparisons of categorical and continuous variables were analyzed by the Chi-Square test and Mann-Whitney U test. Significance was set at p<0.05.

Results: PET/CT was positive in 78/137 pts. In 35 pts (26%) abnormal PET/CT findings consisted of a single area of increased metabolic activity, whereas in the other 43 (31%) multiple hyper-metabolic lesions were found. Twenty/thirty-five pts with single hyper-metabolic spots had confirmed malignant SL: 17 (49%) were BC recurrences and 3 (9%) were new primary tumors other than BC. In the remaining 15 pts, hyper-metabolic SL were consistent with areas of inflammation, past trauma, thyroid struma and physiological ovarian functioning. Eight pts with SL (2: liver, 3: bone, 2: soft tissues, 1: lung) underwent local therapy, which consisted of surgical excision + radiation therapy (4 pts), percutaneous cementoplasty and radiation therapy (2 pts), radiation therapy alone (1 pts) and percutaneous radiofrequency ablation (1 pts). After local treatment 7 pts also received systemic therapy: chemotherapy for 3 and endocrine therapy for 4 pts. Pts with solitary bone lesion also received zoledronic acid. Nine pts received only systemic therapy (4: soft tissues, 2: bone, 2: liver, 1: lung). Median age at the time of diagnosis of SL, histology of primary tumor, hormone receptor status, HER2 status, site of SL and prior exposure to adjuvant chemotherapy did not differ between pts treated with local or systemic therapy. At the time of this analysis,
11/17 pts with confirmed SL had progressed (5 had been treated with local and systemic therapy and 6 with systemic therapy alone), and all the pts were alive.

Discussion: Our retrospective analysis suggests that FDG-PET/CT is a useful tool to identify BC pts with OMD susceptible of local and aggressive treatment, despite the impressive number of solitary non-neoplastic lesions. Our numbers are too small to point out any benefit from the addition of local over systemic treatment, and survival analyses were not significant. Nevertheless, due to growing evidences of a benefit of aggressive treatments for SL, treatment options for pts with OMD cannot leave aside local treatments. However, with the limitation due to small numbers and retrospective design, our data are hypothesis generating, and should be interpreted with caution.

P2-01-01
Withdrawn by Author

P2-01-02
Pharmacological Inhibition of RANKL Attenuates Mammary Tumor Development and Lung Metastases in the MMTV-neu Transgenic Spontaneous Tumor Model.

Background: RANK and its ligand (RANKL), key factors for bone remodeling and metastasis, are crucial for the development of mouse mammary glands during pregnancy. In a mouse model of mammary carcinogenesis induced by the hormone medroxyprogesterone (MPA) and a carcinogen (DMBA), treatment with a RANKL inhibitor RANK-Fc reduces hormone-induced mammary epithelial proliferation, incidence of preneoplasias, and mammary adenocarcinomas. This study assessed the expression of mouse RANK and RANKL in FVB wild-type (WT) and MMTV-neu mice and whether RANKL inhibition could inhibit mammary tumor formation and lung metastases in this model.

Materials and Methods: MMTV-neu mice were treated with the RANKL inhibitor RANK-Fc (10 mg/kg, 3x/week) or vehicle (muFc) beginning at 20 weeks. Mammary tumor formation was determined by palpation and confirmed by histologic examination. The presence of preneoplastic lesions and histotype of tumors was determined on H&E-stained tissues. Entire lungs from tumor-bearing MMTV-neu mice were step-sectioned at 75 μm and assessed histologically for the presence of metastases. Epithelial proliferation was measured by BrdU labeling of the mammary epithelium. The expression of RANK, RANKL, cyclin D1, progesterone receptor (PR), F4/80, FOXP3, and CD3 was determined by immunohistochemistry (IHC).

Results: RANK-Fc treatment of MMTV-neu mice resulted in a significant reduction in total number of mammary tumors and lung metastases quantified at necropsy but did not impact median survival.

Discussion: RANKL is expressed in normal mammary epithelia of MMTV-neu mice but is not detected in any stage of atypia, including MIN, mammary adenocarcinoma, or lung metastases. Selective pharmacological inhibition of RANKL by RANK-Fc treatment attenuated mammary tumor development and lung metastases in the MMTV-neu transgenic spontaneous tumor model.

P2-01-03
Insulin-Like Growth Factor I (IGF) Induced Metastasis Signature Correlates with Poor Prognosis and Decreased Distant Metastasis-Free Survival in Human Breast Cancer.
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The insulin-like growth factors (IGFs) acting via the type I insulin-like growth factor receptor (IGF-1R) regulate tumor growth, survival and metastasis of multiple types of cancers. Agents targeting IGF-1R are currently being tested in phase II/III clinical trials in human cancers. We have previously reported that IGF1R can regulate metastasis independently of primary tumor growth in a model of high risk metastatic breast cancer using the triple negative MDA435/LCC6 metastatic cancer cells, which form spontaneous lung metastases when injected into the mammary fat pad of mice. We have shown that disruption of IGF signaling either with an antibody against IGF1R, AVE1642, or a dominant negative IGF-1R construct, does not affect growth in the mammary fat pad but inhibits pulmonary metastases and colonization of the lungs. Regulation of such a phenotype will not be measured in clinical trials and these findings imply that clinical trials of this therapeutic strategy may need to adjust their endpoints for assessing benefit by taking into account that inhibition of IGF1R signaling may inhibit metastasis but not primary tumor growth.

Here, we sought to develop an IGF induced metastasis signature that may be useful in selecting patients that could benefit from IGF1R targeted therapy and determine if changes in expression of the genes in the signature can be useful in monitoring response. Serumstarved LCC6-WT cells (with wild-type functional IGF1R) and LCC6-DN (cells with dominant negative IGF1R) were treated with IGF-I, AVE1642 (fully human antibody against IGF1R that inhibits metastasis but not tumor growth in the mammary fat pad), or AVE1642+ IGF-I for 4 and 24 hours and microarray analysis was performed using the U133 Plus 2.0 human arrays with 47,000 transcripts. IGF-I regulated over 100 transcripts in LCC6-WT cells compared to IGF-I in LCC6-DN cells. Over 400 transcripts were basally differentially expressed in LCC6-WT cells compared to LCC6-DN cells. Array data were also analyzed to determine genes regulated by IGF-I signaling in LCC6-WT cells compared to untreated LCC6-WT cells and if disruption of signaling with AVE1642 affected genes whose expression was regulated by IGF-I signaling. A total of 263 transcripts were regulated by IGF signaling in LCC6-WT cells compared to untreated cells and AVE1642 treatment reversed the regulation of all transcripts induced by IGF-I in LCC6-WT cells.

53 of the most significantly regulated genes were used to query two different human breast tumor datasets to determine if genes in IGF signaling induced metastasis signature are associated with lymph node metastasis and poor prognosis in human tumors. IGF regulated metastasis signature genes were expressed in human tumors in the van’t Veer and Wang datasets. Further, IGF activated metastasis.
signature was correlated to decreased survival and decreased distant metastasis-free survival in breast cancer patients. Our results suggest that presence of IGF-induced metastasis signature in patients confers prognostic value and identifying IGF-driven metastasis signatures may be useful to identify and monitor patients who could benefit from IGFR1 targeted therapy for inhibition of metastatic disease.

P2-01-04
The Post-Partum Diagnosis of Pregnancy Associated Breast Cancer Confers an Increased Risk for Metastasis without Increased Incidence of Poorer Prognosis Biologic Subtype.
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**Background:** All women who complete a childbirth have an increased risk for the subsequent breast cancer regardless of age at their first birth. In younger women, this period truncates 10 years later and pregnancy then becomes protective, whereas in older mothers the risk persists. Moreover, breast cancers diagnosed subsequent to a recent childbirth have an increased risk for metastasis and death. However much of the existing data is confounded by inclusion of cases diagnosed during pregnancy. **Hypothesis:** Our preclinical models of pregnancy associated breast cancer (PABC) has identified the “involution hypothesis”, where the normal physiologic post-partum breast involution is the pregnancy-related event promotional of breast cancer growth, invasion and metastasis. We have performed a retrospective cohort study, restricted to postpartum PABC and controls, to fully characterize post-partum PABC by biologic subtype and known predictors for metastasis. Also we identify if the specific period of post-partum PABC has a higher risk for metastasis then non-PABC, as predicted by our animal model studies. **Methods:** We performed a retrospective cohort of 527 cases of breast cancer diagnosed at age 45 or younger from 1981-2011. Pregnancy status was identified from medical records and defined as nulliparous (n=107), PABC (up to 5 years post-partum of last childbirth, n=114), and Later Parous (>10 years post-partum, n=239). Clinicopathological characteristics were obtained from pathology records. Outcomes data obtained through the University of Colorado Tumor Registrar. Hazard ratios of risk of metastatic recurrence of nulliparous and PABC groups were analyzed using a Cox Proportional Hazards Model and time to metastatic recurrence was analyzed using standard Kaplan Meier curves. **Results:** Compared to nulliparous cases, PABC cases were more frequently diagnosed at Stage IV (11.4% vs. 4.9%), T3 tumor size (17.3% vs. 13.1%), poorly differentiated (36.6% vs. 49.0%), lobular histology (4.5% v. 1.0%), with lymphovascular invasion (30.4% vs. 24.5%) and with involved lymphnodes (57.6% v. 48.6%). No differences were seen between Luminal A, Luminal B, Her 2 and Triple Negative subtypes. Average follow-up was 3.4 years for nulliparous, 3.5 for PABC and 4.6 for Later Parous. Survival analysis revealed a cumulative 5-year metastatic recurrence free probability of 80.6% for nulliparous, 68.5% for PABC and 80.5% for Later Parous. Compared to nulliparous, PABC had greater than a three times higher risk for subsequent metastatic recurrence (HR 3.29, 95% CI: 1.25-8.63). No significant difference in the risk of metastatic recurrence for Later Parous compared to nulliparous was observed (HR: 1.079, 95% CI: 0.46-2.55). **Conclusion:** Post-partum PABC is characterized by higher stage at diagnosis but without enrichment for poorer prognosis biologic subtypes. Post-Partum PABC has an increased risk for metastasis within five years of diagnosis, with an overall 3.29 times higher risk of a metastatic recurrence. These data support our hypothesis that the post-partum window, and the associated role of naturally occurring involution, may be promotional of tumor growth, invasion and metastasis as previously identified in our animal models.

P2-01-05
Integrin αvβ6 Mediates HER2-Driven Invasion in Breast Cancer.
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We have shown strong expression of integrin αvβ6 reduces the 5 year survival of HER2-positive breast cancers from 66% (HR 1.84) for moderate/low αvβ6 expressing to 54% (HR 2.18) in cases with strong expression (2063 cases, unpublished). This is in direct comparison to HER2/αvβ6-double negative cases, where strong αvβ6 expression reduces survival from 86% (HR 1.00) to 77% (HR 1.20). The biological mechanism underlying these observations was investigated in two isogenic breast cancer models: MCF-7-neo-1 and MCF-7/HER2-18 (a gift from Prof. M-C. Hung, USA) and MCF10A and MCF10A.CA1a.

Flow cytometry showed MCF-7/HER2-18 expressed high levels of both HER2 and αvβ6 whereas MCF-7-neo-1 expressed low levels of both receptors. MCF10A and MCF10A.CA1a both expressed high levels of αvβ6 whereas only MCF10A.CA1a expressed elevated levels of HER2. In charcoal-stripped (cs)-serum, comparing MCF-7/neo-1 and MCF-7/HER2-18, HRGβ1 (1µM), which stimulates HER2/HER3 heterodimers, increased proliferation by 50.2%±9% (P=0.048) and 66.2%±5.5% (P=0.003), in MCF-7-neo-1 and MCF-7/HER2-18 cells respectively. In contrast, Herceptin reduced proliferation by 32.3%±13.4% (P=0.003) and 15.2%±3.4% (P=0.028), respectively. MCF10A and MCF10A.CA1a proliferation remained unchanged with HRGβ1 treatment and antibody-blockade of αvβ6 did not affect proliferation of any cell line. (NB, in complete serum there was no effect on proliferation of any of the above treatments). Invasion through Matrigel of MCF-7/HER2-18 was inhibited by antibody blockade (10µg/ml) of αvβ6 (mAb 10D5; 38.6%±20.8%, P=0.005) or HER2 (Herceptin, 10µg/ml; 40.1%±28.6%, P=0.01). The same trend was observed in MCF10A.CA1a invasion (83%±30.2% (P=0.025) with 10D5 and 80.4%±8.7% (P=0.022) with Herceptin). Combination of both antibodies had no additional effect.

siRNA knockdown of αvβ6 or HER2 in MCF-7/HER2-18 and MCF10A.CA1a cells also reduced invasion to a similar extent as the blocking antibodies. This suggests that HER2 driven breast carcinoma invasion is mediated by αvβ6. To investigate this further HER2/3 was stimulated with HRGβ1, which consistently increased invasion by 111.5%±35.4% (P=0.011) in MCF-7/HER2-18 cells and by 57%±34% (P=0.042) in MCF10A.CA1a cells; an increase that was abrogated by co-treatment with 10D5 or Herceptin.

To determine the mechanism through which HER2 and αvβ6 cooperate we examined several signalling pathways. Analysis of total or activated Akt, ERK1/2, e-Jun or Src in the MCF-7 model showed no changes. However, elevated total and phospho-Stat3 in MCF-7/HER2-18 were observed and siRNA knockdown, or small-molecule inhibition, of Stat3 suppressed invasion of MCF-7/HER2-18 cells (54.5%±27.3% (P=0.008) and 55.3%±33.3% (P=0.01) respectively), possibly suggesting that activation of Stat3 may link αvβ6 and HER2 co-operative signalling in this model. Interestingly, Akt was constitutively phosphorylated in MCF10A.CA1a cells and, moreover, 10D5 reduced these levels suggesting αvβ6 may influence HER2 signalling via Akt in these cells.

These data confirm HER2-driven invasion is αvβ6-mediated and
provide a mechanistic explanation for our clinical observations. We suggest HER2 and avp6 should be considered as dual targets for future therapy of some breast cancers.

P2-01-06
NSAID Analgesic Ketorolac Used Perioperatively May Suppress Early Breast Cancer Relapse: Something for Nothing in Breast Cancer

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Background: Over a decade ago, we were confronted with data that showed an unexpected bimodal pattern of hazard of relapse among early stage breast cancer patients treated by mastectomy without any adjuvant systemic treatment. This pattern has now been identified in 20 independent databases from US, Europe and Asia. There is an early peak of relapses at 18 months, a nadir at 50 months and a broad second peak extending from 60 months to over 15 years. Fifty to eighty percent of relapses, the proportion increasing with primary tumor size, reside within the first peak. This pattern was not explainable by accepted theories. We proposed based on computer simulations that the broad second peak relapses result from steady stochastic progressions from single dormant malignant cells to avascular micrometastases and then on to growing deposits. To explain the first peak, we had to postulate that something happened at about the time of surgery to provoke sudden exits from dormant phases to active growth and then to detection. There was a particularly sharp early relapse mode within 10 months for premenopausal patients with positive nodes that appeared to be surgery-induced angiogenesis of dormant avascular micrometastases. We have been able to explain a wide variety of breast cancer observations with this hypothesis. These include high effectiveness of adjuvant chemotherapy only for premenopausal node positive women and why early detection works better for women age 50-59 than for women age 40-49.

Methods and Materials: Forget et al reported data from a retrospective study of 327 consecutive patients comparing various perioperative analgesics and anesthetics in one Belgian hospital and one surgeon. Patients were treated with mastectomy and conventional adjuvant therapy. Follow-up is average 27.3 months with range 13-44 months.

Results: NSAID ketorolac, a common analgesic used in surgery, produced far superior disease-free survival in the first 5 years after surgery. The expected prominent early relapse peak is all but absent. These results are superior to any adjuvant therapy we have examined. Discussion: If this observation holds up to further scrutiny, it could mean that the simple use of this safe and effective anti-inflammatory agent at surgery might eliminate early relapses. Concerning a possible mechanism, since ketorolac is the only anti-inflammatory agent among the analgesics studied, our attention is drawn to inflammation. Might this imply that transient systemic inflammation accompanying surgery is part of the metastatic tumor seeding process? Inflammation can be a very rapidly occurring effect since bump and redness following a mosquito bite occur within seconds. It is well established that many cancer patients have circulating cancer cells. Data show a surge in circulating epidermal cells after primary breast cancer surgery but that intriguingly occurs days later. Blood speed in capillaries is 0.03cm/sec which would make leaky capillary venules a very efficient way for circulating cancer cells to enter tissue. From our simulations the few relapses in the ketorolac data look like the leading edge of the late broad peak - viewable for the first time.

P2-01-07
Proto-Oncogene PELP1 Promotes Metastasis through miR-200 Dependent Pathway.


Background: Endocrine therapy is the most important component of adjuvant therapy for patients with early stage estrogen receptor (ER)-positive breast cancer. Despite positive effects of hormonal therapy, initial or acquired resistance to endocrine therapies frequently occurs and tumor recurs as advanced metastatic disease. Accumulating evidence suggests that ER-coregulators play an essential role in cancer progression and that metastatic tumors have increased expression of coregulators. Proline glutamic acid rich protein (PELP1) is an ER coregulator, its expression is upregulated during breast cancer progression to metastasis. PELP1 is an independent prognostic predictor of shorter breast cancer specific survival and its elevated expression positively associates with markers of poor outcome. The objective of this study is to examine the mechanism and significance of PELP1 mediated signaling in breast cancer metastasis.

Methods: To examine the significance of PELP1 in metastasis, we have used two ER+Ve (ZR75, MCF7) and one ER-ve MDA-MB 231 model cell that either stably express PELP1 or PELP1 shRNA. MCF7 and ZR75 cells were used as controls. Role of PELP1 on metastasis was studied using Boyden chamber, wound healing, invasion, MMP and reporter gene assays. Epithelial to Mesenchymal Transition (EMT) real time qPCR Array (Super array) was used to identify PELP1 target genes. Whole genome based microarray analysis was performed to identify PELP1 regulated microRNAs. miRNA mimetics and antamiRs were used to establish the mechanism. Nude mice based assays were performed to study the role of PELP1 on in vivo metastasis.

Results: Boyden chamber and wound healing assays showed PELP1 down regulation substantially affect migration of both ER+ve and ER-ve cells and showed alterations in the expression of the EMT markers. Focused microarray studies identified PELP1 modulate expression of eight genes involved in the EMT (including Snail, Twist, ZEB1 and MMPs). In xenograft assays, overexpression of PELP1 in non-metastatic cells increases their propensity for metastasis in vivo, while PELP1 knockdown in metastatic model cells decreased in vivo metastatic potential. Whole genome microRNA array analysis revealed that miR 200a and miR141 were upregulated in cells expressing PELP1-shRNA compared to control shRNA expressing cells. Accordingly, over expression of PELP1 in low metastatic model cells decreased expression of miR200a and miR141. Mechanistic studies showed PELP1 down regulate expression of metastasis suppressive microRNAs (miR200a and miR141) by promoting chromatin modifications. Ectopic expression of miR200a and miR141 mimetic decreased PELP1 mediated metastatic functions.

Conclusions: These results suggest that PELP1 play a role in breast cancer metastasis by promoting cell motility/EMT by modulating microRNA expression and blockage of PELP1 axis has potential to reduce metastasis potential of breast cancer cells. Understanding how NR coregulator PELP1 plays a role in metastasis will be useful in maximizing treatment opportunities for metastatic breast cancer. This study is funded by DOD grant.

P2-01-08
Withdrawn by Author
P2-01-09
Gene Expression Alterations in the Lymph Node Microenvironment in Response to Successful Metastatic Colonization.
Ellsworth RE, Valente AL, Kane JL, Ellsworth DL, Shriver CD, Henry M. Jackson Foundation, Windber, PA; Windber Research Institute, Windber, PA; Walter Reed Army Medical Center, Washington, DC

Background: Breast stroma is known to play an active role in tumorigenesis, undergoing both phenotypic and molecular changes to facilitate and promote tumor development and growth. The metastatic microenvironment also plays a role in successful colonization; however, the genetic changes in these secondary microenvironments associated with metastasis are not well described.

Methods: Women with invasive breast cancer with at least one lymph node with macrometastases and one lymph node with no detectable metastases were identified from the Clinical Breast Care Project. Lymph node tissue was microdissected from both the metastatic lymph node microenvironment and negative nodes and hybridized to U133A 2.0 gene expression arrays. Differential expression was detected using Partek® Genomics Suite™6.5 using a cutoff of P<0.001, >2-fold change.

Results: Nineteen genes were differentially expressed between negative lymph nodes and lymph node tissue microdissected from lymph nodes with metastatic tumors. Eleven genes, including EPCAM, KRT19 and MUC1 were expressed at significantly higher levels in lymph node tissue from metastatic lymph nodes while eight genes, such as CXCL2 and CXCL5, were expressed at significantly higher levels in negative lymph nodes. Results have been validated in external sample sets for AZGP1, CLEC4M, CXCL2, EPCAM, MUC1, PIP and TFPI.

Conclusions: Lymph node tissue differs in gene expression between those harboring metastatic tumors and those without metastasis. Genes expressed at higher levels in lymph nodes with macrometastases are involved in tumorigenesis, suggesting that like the breast stromal microenvironment, the metastatic microenvironment undergoes cross-talk with the tumor cells. In addition, a cluster of genes involved in immune function are expressed at lower levels in metastatic lymph nodes, suggesting that suppression of proper immune response may be required for successful metastatic colonization.

P2-01-10
Blockage of Autocrine TGF-b Signaling Reduces Tumorigenicity and Lung Metastasis in a Murine Breast Cancer Cell Line, NMuMG-ST.
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Transforming growth factor beta (TGF-b) is known to have a dualistic role in breast cancer cells, acting as a tumor suppressor or a tumor promoter. This dualistic property varies in different cells and at different progression stages. Studies show that autocrine TGF-b is necessary for the growth and survival of MDA-MB-231 cells, and was also known to enhance the survival of MCF-7 cells. However, the role of autocrine TGF-b in spontaneously transformed cells is still less understood. We have retrovirally transduced a dominant negative TGF-b Type II receptor (DN RII) into a spontaneously transformed murine mammary gland epithelial cell line NMuMG-ST (DN RII cells). The expression of DN RII reduces TGF-b sensitivity of NMuMG-ST cells in a gene transcription assay and a growth inhibition assay. Interestingly, the autocrine TGF-b supports the survival of NMuMG-ST cells, although the exogenous TGF-b inhibits the growth of NMuMG-ST cells. Moreover, more apoptosis were observed in the DN RII cells compared to the control cells in both in vivo and in vitro experiment. We found that AKT and ERK pathway mediates autocrine TGF-b’s survival signal. Results of western immunoblot for several stem cell markers and mammosphere formation assay suggest that autocrine TGF-b signaling is essential for the maintenance of stem like cell subpopulation in NMuMG-ST cells. By using immunocytostaining, we found that the DN RII cells expressed more E-cadherin and less Vimentin when compared to the control NMuMG-ST cells, which indicate that blocking autocrine TGF-b signaling pathway could inhibit the process of EMT in NMuMG-ST cells. Notably, when the cells were inoculated orthotopically in nude mouse mammary glands, tumors formed by DN RII cells grew at a similar rate as those formed by control cells, but caused fewer lung metastases. Our finding identified that the autocrine TGF-b signaling is important for the survival and metastatic ability of NMuMG-ST, which could provide an important foundation for further investigation on the role of autocrine TGF-b signaling in breast cancer.

P2-01-11
Fish Oil Targets miR-21 To Increase PTEN Expression for Inhibition of Bone Metastatic CSF-1 in Breast Cancer Cells.
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Current therapy for the breast cancer patients is aimed at treating the cancer mainly at the primary site with limited ability to block metastasis. We have recently shown that fish oil (FO) diet significantly prevents MDA MB-231 breast cancer cell (MDA) metastasis to bone in a mouse model. Colony stimulating factor-1 (CSF-1) is one of major osteoclastogenic cytokines responsible for osteolytic metastasis of breast cancer. Quantitative RT-PCR (qRT-PCR) analysis showed significant inhibition of CSF-1 mRNA expression in fish oil fed mice xenograft tumor samples and docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), two ω-3 fatty acids in fish oil, inhibited CSF-1 mRNA and protein expression in MDA cells. A positive correlation was observed between expression of CSF-1 and the bone metastatic potential of different breast cancer cells. We have demonstrated recently upregulation in PTEN expression by FO diet as a mechanism of breast tumor growth arrest in mice. Over expression of PTEN inhibited migration of MDA cells. PTEN is a negative regulator of PI3 kinase/Akt signaling. We investigated the mechanism of CSF-1 expression by breast cancer cells. Cotransfection of the CSF-1 promoter-driven luciferase plasmid (CSF-1-Luc) in MDA with PTEN or dominant negative (DN) PI3 kinase or DN Akt, inhibited the transcription of CSF-1, indicating requirement of PI3 kinase/Akt signaling. Several oncogenic microRNAs including miR-21 have been shown to induce metastasis of cancer cells. Fish oil fed mice tumors and DHA- and EPA-treated MDA cells significantly blocked miR-21 expression in MDA. PTEN mRNA 3'UTR contains miR-21 recognition element. Over expression of miR-21 inhibited PTEN 3’UTR-Luc reporter activity but it was increased by downregulation of miR-21, indicating functional targeting of PTEN mRNA by miR-21 in MDA. Similarly, expression of either miR-21 or miR-21 Sponge (plasmid-coded anti-miR-21 sequences) in MDA significantly suppressed or increased PTEN protein, respectively. Moreover, DHA-increased PTEN expression is reversed by over expression of miR-21 indicating a critical regulation of PTEN by miR-21. miR-21 over expression increased migration of MDA cells and partially reversed DHA- or EPA-mediated inhibition of migration. Since PTEN
regulates expression of CSF-1, we examined the involvement of miR-21. Expression of miR-21 enhanced while miR-21 Sponge reduced expression of CSF-1 mRNA and its transcription. Furthermore, DHA-inhibited CSF-1 mRNA expression is reversed by over expression of miR-21. Together these results uncover a novel function of fish oil, which beheads miR-21 signaling to dampen expression of CSF-1 thus preventing osteoclastogenesis and osteolytic lesions in breast cancer.

P2-01-12
Determining the Molecular Signature That Drives Breast Cancer-Induced Brain Metastasis in a Mouse Xenograft Model.

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Breast cancer (BCa) is the most common malignant disease in women in U.S. and the mortality in BCa patients is due to the metastasis of the disease from primary site. Brain metastasis accounts for nearly 20% of all metastases in BCa. It is the most feared complication of BCa because of very few effective treatment regimens available, leading to lower survival rate in patients. The exact molecular mechanism for metastases of BCa into brain is unknown. Rodent model systems have been reported for brain metastasis in BCa but the current models for brain metastasis have limitations. Therefore, there is a need of efficient model system that can significantly contribute towards our understanding of different factors from both host and tumor leading to brain metastasis.

Previously we reported isolation and characterization of estrogen independent B6TC cells that are derived from the stable spontaneous fusion of MDA-MB-231/GFP/Neo and ZR-75-1/GFP/puro in mouse bone marrow microenvironment. This chimeric B6TC has propensity to metastasize to brain when inoculated through intracardiac (I.C.) route, and express stem cell-like features. In the present study using B6TC, we have developed an efficient and novel mouse model for studying BCa-induced brain metastasis. We have generated three cell lines from B6TC through three successive rounds of inoculation in mouse and subsequent isolation of brain metastatic cells. Each round of selection enhanced the brain metastatic propensity. An initial microarray analysis identified genes like MMP1, HB-EGF, ST3GAL1, PTGS2, ITGA3, and CXCR4, which showed significant up-regulation in B6TC compared to its parental metastatic MDA-MB-231 or non-aggressive ZR-75-1 cells. These genes are implicated in metastasis regulation. A second round of RNA microarray was performed with three sublines of B6TC with successively enhanced brain metastatic propensity to identify unique potential brain metastatic genes showing gradual up or down-regulation over generations. From analysis of the gene expression profiles, apart from potent brain metastatic genes detected earlier, we also identified some molecular pathways, including TGF beta signaling pathway that are associated with enhanced brain metastasis. The B6TC model is novel for studying the molecular mechanism of brain metastasis, as in this model, apart from experimental metastasis in brain through I.C. route, cells show spontaneous metastases to the brain from the primary tumor and this unique feature will enable us to study the mechanisms of the early steps of brain metastasis progression. Further analyses to find out common miRNAs that are over or under-expressed in the sublines over successive generations and determination of gene targets of miRNA are underway. This study will not only provide valuable insight into molecular mechanism of BCa-induced brain metastasis but also lay the foundation to identify novel prognostic and therapeutic markers of brain metastases, leading ultimately to the discovery of novel molecularly targeted drugs to prevent and eradicate BCa metastasis initiation, progression and recurrence.

P2-01-13
Impact of Erlotinib on MSC-Mediated TIC Expansion and EMT.

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Background: Recently, we have demonstrated that mesenchymal stem cells (MSC) and MSC secreted factors (MSC-CM) have a profound effect on tumor initiating cells (TIC) enriched mammosphere formation and latency of tumor xenografts formation from breast cancer cell lines. Furthermore these interactions increased the expression of epithelial mesenchymal transition (EMT)-associated proteins which are associated with tumor cell invasion and metastasis as well as the TIC phenotype. (Klopp, A. H. et al., 2010, PLoS One. 5, e12180). Our data suggest that the presence of MSC in the tumor microenvironment may increase metastases by conferring stem progenitor cell biology on more differentiated non-metastatic cells. In addition, preliminary data suggested MSC-CM upregulated EGFR signaling in breast cancer cells. Therefore, we hypothesized that inhibiting EGFR signaling with erlotinib (tyrosin kinase inhibitor) can suppress MSC-mediated TIC expansion and EMT.

Methods & Results: In order to demonstrate that erlotinib inhibits MSC-CM promoted expansion of TIC, we cultured breast cancer cells lines (SUM149, SUM159, SUM190, MDA-IBC3 and MCF-7) in anchorage independent conditions with MSC-CM and treated them with increasing concentrations of erlotinib. The efficiency of mammosphere formation was examined after 5 days. We found that erlotinib inhibited MSC mediated increase in mammosphere formation in triple negative cell lines SUM149 and SUM159, and HER2-positive cell lines SUM190 and MDA-IBC3, but not in ER-positive, erlotinib resistant MCF-7 cells. Furthermore, we evaluated the impact of erlotinib on cell cultures grown with breast cancer patient-derived fluids, such as seroma and malignant pleural effusions. We observed that the effect of erlotinib on mammospheres formation was attenuated by both types of patient fluids.

Discussion: Patients with triple negative breast cancer have the highest rates of metastases and no available targeted therapies for treatment. EGFR is expressed in a significant proportion of triple negative breast cancers, and recent clinical and preclinical studies suggest that EGFR may contribute to the metastasis or aggressiveness of triple negative breast cancer. Here we demonstrate that host and environmentally-derived factors are critical for determining resistance to erlotinib. In vivo studies regarding the ability of erlotinib to prevent MSC-enhanced TIC survival and metastases are underway.

P2-01-14
High Level of CD44 Expression and Soluble CD44 Secretion Mediate Trastuzumab Resistance in HER2 Positive Breast Cancer.

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Background: Overexpression of HER2 occurs in 15–25% of invasive breast cancer. Trastuzumab, a humanized monoclonal antibody targeting the extracellular domain of HER2, markedly improves disease-free and overall survival in patients with HER2-positive breast cancer. However, resistance to trastuzumab develops in most patients with HER2-overexpressing metastatic breast cancer. Breast cancer stem cells have been shown to express HER2, although its role in the
development or maintenance of stem cells is not well characterized. CD44 is one of the proposed markers for breast cancer stem cells. We investigated the role of CD44 in acquired trastuzumab resistance in HER2 positive breast cancer.

Methods: CD44 mRNA expression was measured by quantitative real-time PCR. The proportion of CD44 positive cells was measured by fluorescence-activated cell sorting (FACS). Soluble CD44 level was detected by enzyme-linked immunosorbent assay (ELISA). In vitro tumorigenicity was evaluated by soft agar colony formation assay. Cell invasion was tested by using a matrigel invasion assay. In vivo tumor growth was evaluated using a mammary fat pad xenograft mouse model.

Results: CD44 mRNA expression, CD44 positive cell population by FACS, and CD44 secretion were significantly increased in both SKBR3 derived trastuzumab resistant SKBR3 clone 3 and BT474 derived trastuzumab resistant HR20 cells compared to parental, trastuzumab sensitive cells. Consistent with the increase in CD44, trastuzumab resistant cells showed an increase in soft agar colony formation and cell invasion, which were blocked by siRNA-induced downregulation of CD44 expression. Tumor growth and incidence were enhanced in mice injected with trastuzumab resistant HR20 cells compared to the mice injected with trastuzumab sensitive parental BT474 cells. Knock-down of CD44 expression by the transfection with CD44-specific siRNA suppressed in vivo tumor growth in the mice injected with trastuzumab resistant HR20 cells. Finally, we found high serum CD44 levels correlated with advanced tumor progression in HER2 positive breast cancer.

Conclusion: Our findings indicate that high levels of CD44 mediate trastuzumab resistance in HER2 positive breast cancer cells as well as tumor progression in mice. Circulating CD44 is prognostic in patients with HER2 positive breast cancer. CD44 may be a therapeutic target for HER2 positive breast cancer.

P2-01-15
RT-PCR Gene Profiling for the Prediction of Bone Metastases in Primary Breast Cancer:
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Background: Patients with breast cancer frequently develop bone metastases, which are responsible for high morbidity and mortality. It is known that numerous molecules are involved in the bone metastasization process and that only cancer cells with a specific molecular profile are capable of reaching and colonizing bone tissue. The early detection of patients with a high probability of relapsing to bone could be used to select candidates for tailored therapy with bone-specific drugs such as bisphosphonates or RANK-L inhibitors. The aim of the present study was to identify a pattern of tissue markers from primary breast cancer capable of predicting bone metastatization.

Material and methods: The expression of different markers was retrospectively analyzed in frozen breast cancer tissue samples from 40 patients comprising 15 cases with no evidence of disease (NEDP), 15 with bone metastases (BMP), and 10 with visceral metastases (VMP). Twelve transcripts were analyzed by Quantitative Real time PCR: trefoil factor 1 (TFI), DKK1, bone sialoprotein (IBSP), heparanase (HPSE), osteopontin (SPP1), Agr2, SPARC, CTGF, COMP, follistatin (FST), osteoprotegerin and RANK. The predictive accuracy of each marker was calculated using receiver operating characteristic (ROC) curves.

Results: Analysis of marker expression in the 3 different subgroups showed twofold higher median values of COMP and HPSE in BMP with respect to either NEDP or VMP (no significant difference). Furthermore, TFF1 median value was about sevenfold higher in BMP than in the other 2 subgroups (p=0.05). The area under the curve (AUC) was 0.73 for TFF1 and 0.62 for COMP and HPSE. Considering markers as dichotomous variables, TFF1 expression in BMP reached 67% compared to 21% and 20% in NEDP and VMP, respectively. In dichotomous variable analysis, the most important result was observed for TFF1; in particular, TFF1 positivity was observed in 67% of cases in the BMP subgroup compared to only 21% and 20% in NEDP and VMP, respectively. The combination analysis of TFF1 and other markers showed that positivity to at least one of the three markers, TFF1, DKK1, or IBSP, was observed in 80% of cases for the BMP group, and in only in 21% and 20% of cases in NEDP and VMP subgroups (p=0.041). Another interesting marker was RANK, although sensitivity was fairly low (12%), specificity reached 100% in both control groups.

Conclusions: Our preliminary study identified a RT-PCR gene expression pattern in primary breast cancer that could predict patients destined to bone relapse. Such patients could consequently benefit from adjuvant treatment with bone-targeted therapy.

P2-01-16
Dissecting the Cellular Mechanism of Induction and Sustenance of Dormancy by Rho GTPases in Breast Cancer.
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Tumor dormancy has been a very real problem in breast cancer survivors where recurrences are common and usually fatal. Molecular mechanisms involved in this process are often poorly understood and are a hot spot in breast cancer research today. We have found that RhoGTPases, specifically RhoC, is hyperactivated in low metastatic dormant breast tumor cells while RhoA is downregulated. We also found RhoC causes phosphorylation of c-jun NH2 terminal kinase (JNK) in these cells which in turn activates the process of autophagy, a possible mode of cell survival in dormant tumor cells. Modulating RhoC affected phosphorylation of JNK. Also, abrogating JNK by a pharmacological inhibitor SP600125 inhibited expression of the autophagy marker LC3B-II. Autophagy genes like Atg 5 and Atg12 were expressed along with the autophagy marker LC3B-II. On inhibiting autophagy by a specific inhibitor 3-Methyladenine (3-MA), cells did not readily undergo apoptosis but on stressing them doubly with LY294002, an inhibitor of Class I PI3K, although sensitivity was fairly low (12%), specificity reached 100% in both control groups.

Conclusions: Our preliminary study identified a RT-PCR gene expression pattern in primary breast cancer that could predict patients destined to bone relapse. Such patients could consequently benefit from adjuvant treatment with bone-targeted therapy.
P2-01-17
L1-CAM Promotes Adhesion of Breast Cancer Cells to the Endothelium.
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Background: Overexpression of the adhesion molecule L1-CAM (L1) in breast cancer is correlated with nodal involvement, high grading, and a shorter recurrence-free and overall survival, but the mechanism leading to this effect is poorly understood. L1 is also expressed in endothelial cells promoting the interaction between lymphatic cells and endothelia via homophilic L1-L1 interaction. This mechanism might also mediate adhesion of breast cancer cells to endothelia and thus promote metastasis. To examine this likely role of L1, the impact of L1 expression in breast cancer cells on their adherence to human pulmonary microvascular endothelial cells (HPM EC) was investigated and potential L1 ligands on these cells were identified.

Methods: MDA-MB231-Fra2 breast cancer cells expressing high levels of L1 were stably transfected with shRNA vectors to generate two clones with strong L1 downregulation (L1 low clones). Adherence of these clones to endothelial cells was studied in dynamic cell-flow adhesion assays in channels seeded with HPM EC simulating in vivo-conditions. Rolling or adherent cells per minute were counted using CapImage software. For the cell-flow assay HPM EC were activated with TNFa. In order to identify the L1 ligand, endothelial cells were partly pre-incubated with blocking antibodies directed to the potential binding partners L1, ALCAM, ICAM-1 and E-selectin before interaction with tumor cells. In additional experiments, flow chambers were coated with recombinant L1-CAM, ICAM1, ALCAM or E-selectin, and the adherence of MDA-MB231 cells with high or low L1 expression to these proteins was investigated.

Results: Adhesion of MDA-MB231 cells to activated HPM EC was significantly higher in L1 high cells compared to L1 low clones, where the number of adherent cells was only 40-50% of the L1 high control (p=0.025; p=0.035). Blocking experiments showed that the adherence of L1 high cells could be reduced by antibodies directed to ALCAM (p=0.0007), but not to ICAM1. Anti-L1 antibodies had a significant effect only in passages of endothelial cells which showed L1 expression, whereas E-selectin blocking strongly diminished adherence of breast cancer cells irrespective of their L1 expression. In addition, L1 low clones showed significantly lower adhesion to recombinant L1, ALCAM and E-selectin proteins compared to the parental cells.

Conclusion: Our experiments indicate that L1 expression in breast cancer cells leads to an increased adherence to activated endothelial cells via homophilic (L1-L1) or heterophilic (L1-ALCAM) interactions. This mechanism is a possible explanation for the increased metastatic potential and poor prognosis in L1-positive carcinomas observed in vivo. Our results suggest that this adhesion molecule might be a suitable target for therapeutic interventions.

P2-01-18
Cancer Stem-Like Cells in Breast Cancer Progression.
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Background: increasing evidences support the presence of cancer stem-like cells (CSCs) within breast carcinomas. A subset of cells enriched for CSCs within the tumor can be identified by flow cytometry. It has been supposed that CSCs component is responsible for tumor resistance and metastasis. However, the analysis of such population has never been evaluated during breast cancer progression.

Patients and Methods: We prospectively included 98 women with histologically proven breast carcinomas to assess the presence of CSCs within the primary tumor. According to clinical stage (CS), five patients were CS 0 (palpable DCIS), 16 CS 1, 20 CS II and negative lymph nodes (IIA-), 20 CS II and positive lymph nodes (IIA+), 6 IIIa, 17 IIb, IIIc and 2 CS IV. Additionally, ten lymph node metastases from IIA- patients were analyzed. Invasive ductal carcinoma (IDC) was observed in 79 patients. We excluded from the analysis patients with non ductal histology (n=11). The percentage of CD44+/CD24- (n= 79), ALDH1+ (ALDEFLUOR®) (n= 29), ABCG2+ (n= 44 ) and CXCR4+ (n= 46 ) cells within ESA+ cells was determined by flow cytometry in fresh sampled tumors. The mammosphere assay was studied in 14 samples. The relationship between cytometry analyses and clinical and pathological features was analyzed.

Results: The median prevalence of CD44+/CD24- cells within ESA+ population was 1.63% in stage 0, 2.39% in stage 1, 0.42% in stage II, 8.15% in stage IIA+ and 0.52% in stage III (p=0.005). The CD44+/CD24- cell population was analyzed in 10 lymph node metastasis and, the median prevalence of this population within ESA+ cells was 4.7%. There was no variation of median prevalence of ALDH1+, CXCR4+, ABCG2+ and the median number of spheres among clinical stages (p=0.1, p=0.8, p=0.8, respectively). We didn't observe any association among the expression of, ABCG2+, CXCR4+ with histological grade and the expression of ER and PgR. The median percentage of CD44+/CD24- cells was higher (2.39% vs 0.41%) in ER positive tumors (p=0.003). The median percentage of ESA+ CXCR4+ cells was higher (42.5% vs 9.7%) in HER2- tumors. There was no correlation between the studied markers and the patients' age and clinical tumor diameter. The results are summarized in Table 1.

Discussion: We found a significant variation in CSCs population during breast cancer progression. Our data show a gradual increase in CD44+/CD24- cells within ESA+ population from stage 0 to stage IIA+ and a significant decrease in stage III tumors. This observation supports the hypothesis that a population of CSCs could be associated with breast cancer progression.
**P2-01-19**

Expression of Receptor Like Protein Tyrosine Phosphatases mu (PTPRM) in Breast Cancer and the Biological Effects of PTPRM on Breast Cancer Cells.

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Introduction:
Protein tyrosine phosphatases (PTPs) play a major role in many cellular functions including cell survival, proliferation, differentiation and motility. The receptor-like protein tyrosine phosphatase mu (PTPRM) has a similar structure to cell-cell adhesion molecules and has been shown to exhibit homophilic binding and confer cell-cell adhesion in cells including epithelial and cancer cells. However, the role played by PTPRM in breast cancer remains unclear. The present study investigated the expression of PTPRM in breast cancer and the biological impact of PTPRM on breast cancer cells.

Methods:
Expression of PTPRM protein and gene transcript was examined in a cohort of primary breast cancer tumours. The association of PTPRM transcripts level and pathological and clinical aspects was then analysed. Expression of PTPRM transcript in breast cancer cells were knocked down using a specific anti-PTPRM transgene. The impact of PTPRM knockdown on breast cancer was evaluated using a series of in vitro cell models and in vivo tumour model.

Result:
PTPRM protein was seen generally distributed in the breast tissues, including tumours and normal background tissues. A stronger staining was revealed in cytoplasm of both mammary epithelia and cancer cells in comparison with the surrounding stromal cells. A significantly decrease was seen in poorly differentiated tumours (92.5±279), and moderately differentiated tumours (288±110), compared with that of well differentiated (472±114), p=0.011 and p=0.031 respectively. PTPRM transcript levels were decreased in patients died from breast cancer. The expression of PTPRM transcripts was significantly associated with disease free survival. Patients with lower expression of PTPRM had shorter survival compared with those with high level of PTPRM expression (p=0.029). Knocking down PTPRM from breast cancer cells significantly increased cell adhesion compared with control cells (p=0.001). Likewise, the knockdown also marked increase the rate of cell migration (102.9±11.3µm for control vs 115.3±10.5µm for PTPRM knockdown, p=0.05).

Conclusion: The expression of PTPRM transcripts is decreased in poorly differentiated breast tumours and is correlated with poorer long term survival. This is likely to be contributed to by the biological impact of PTPRM on the aggressiveness of breast cancer cells.

**P2-01-20**

Reduced Plakoglobin Expression Increases the Metastatic Potential of Breast Cancer Cells.

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The majority of deaths from breast cancer are a result of metastases, however, little is understood about the genetic alterations underlying their onset. A recent study of human breast cancer biopsies showed alterations of expression of 9 genes in primary tumours that had metastasized compared to benign tumours. Of these, plakoglobin was the most altered, with metastatic tumours showing a 3x decrease in expression compared to benign. Furthermore, expression of plakoglobin is reduced upon autocrine production of human growth hormone, which is associated with increased breast tumour cell invasion in vivo. Plakoglobin codes for the adhesion protein gamma-catenin, an integral part of the cadherin-catenin complex involved in cell-to-cell adhesion. The aim of the current study is to investigate the effects of plakoglobin knockdown on cell proliferation, migration, adhesion and invasion in vitro and in vivo.

The non-metastatic human breast cancer cell lines MCF7 and T47D that express high levels of plakoglobin and gamma-catenin were stably transfected with miRNA to two different regions of the plakoglobin gene, or scramble vector, to produce plakoglobin knockdown and controls for each cell line. Plakoglobin gene expression was monitored by qPCR and gamma-catenin expression by Western blot. Cell proliferation was assessed 24, 48, 72 and 96 hours post seeding; cell migration and invasion were measured over 24 and 48 hours using scratch and modified Boyden Chamber assays. Cell-cell adhesion was assessed by spheroid formation in agar matrix. For in vivo experiments, MCF7 plakoglobin knockdown and control cells were inoculated into the 5th and 10th mammary fat pads of 12-week old female balb/c mice (n=10 per group). Tumour growth was monitored by caliper measurements and local invasion was investigated 6 weeks after tumour inoculation on histological sections. Shedding of tumour cells into the blood stream and evidence of metastatic bone lesions was monitored by flow cytometry and μCT, respectively. Plakoglobin and gamma catenin expression were reduced by more than 80% in all knockdown cell lines used but were unaltered following transfection with the scrambled vector. Furthermore, knockdown of plakoglobin did not effect expression of its binding partner, e-cadherin. Reduced expression of plakoglobin resulted in a more than 3-fold increase in MCF7 and T47D cell proliferation in vitro (p<0.005) and a 2-fold increase in MCF-7 proliferation in vivo (p<0.005), compared with control. In addition plakoglobin knockdown cells showed a 6 – 15 fold increase in invasion through basement membrane (p<0.005), a 2-fold increase in migration (p<0.005) and exhibited reduced cell-to-cell adhesion compared with control cells. Data show that decreased expression of plakoglobin increases the early pro-metastatic behaviour of cells including increased cell proliferation, migration and invasion.

**P2-01-21**

The Biological Influence of Brain Derived Neurotrophic Factor (BDNF) on the Aggressiveness of Human Breast Cancer Cells.

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Introduction: Brain derived neurotrophic factor (BDNF) is a member of the neurotrophin superfamily and has been indicated in the pathophysiology of the nervous system and is important in a number of neurological and psychological conditions. Recently, BDNF has also been shown to play a role in the development and progression of solid tumours myeloma, lung cancer and prostate cancer. It has been recently reported that BDNF is also aberrantly expressed in human breast cancer and that a raised level of BDNF is associated with poor clinical outcome and reduced survival. The present study aimed to investigate the biological role of BDNF in human breast cancer.

Materials and methods: A panel of human breast cancer cells were used in the present study. The expression profile of BDNF was evaluated using RT-PCR. We constructed a set of anti-BDNF transgenes which were used to transfect breast cancer cells in order to generate BDNF knockdown cells (MCF<sub>10AT500</sub>). The impact of BDNF knockdown on growth and cellular migration were evaluated using standard growth assays and ECIS (electronic cell impedance sensing) technology.

Results: BDNF gene transcripts were successfully detected in the
breast cancer cell lines, MCF-7, MDA-MB-231 and ZR 75-1. MCF-7 wild type cells were subject to transfection of anti-BDNF transgenes, followed by the establishment of BDNF knockdown sub-lines and empty vector control cells (MCF-BDNF). Loss of BDNF in breast cancer cells resulted in reduction of cell growth rate (growth rate in MCF-BDNF by day 3 1.60+/-.01, compared with 2.06+/-.04 in MCF-wt, p=0.006). Using electronic cell-cell substrate impedance sensing, it was found that loss of BDNF in breast cancer cells resulted in a significant increase in the rate of cellular attachment and migration (migration rate over 14 h in ohms: MCF-BDNF 6310.125+/-.595.183 compared with MCF-wt 5115.5 +/-422.394, p=0.0003).

Conclusion: When BDNF was stably knocked down in MCF-7 cell lines, the growth and proliferation were decreased. The ECIS results also showed that cell migration was decreased in MCF-7 cells stably transfected with ribozymes for BDNF compared with the vector control. It is concluded that BDNF, a neurotrophic growth factor aberrantly expressed in cancers such as breast cancer, has a profound impact on the cellular behaviour of breast cancer cells and that loss of BDNF is associated with a reduction of the aggressiveness of breast cancer. BDNF is therefore a potential therapeutic target in breast cancer and its effect in human breast cancer requires further investigation.

P2-01-22
Characterization of Four ER-Negative Breast Primary Tumor Dissociated Cultures as a Model for the In Vitro and In Vivo Study of Tumorigenesis, Metastasis and Angiogenesis.
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ER negative breast carcinomas comprise a subtype of tumors for which there are few therapies with successful response. Currently, the use of established cell lines has allowed detailed molecular and cellular studies on cancer mechanisms ranging form stem cells to proliferation, from tumorigenesis to invasion and metastases, and from tumor suppressors to oncogenes. But while cell lines provide a source of homogeneous, propagatable material, a drawback of this model is that most breast cancer cell lines are derived from metastatic lesions that may harbor additional mutations and have little resemblance with the primary tumor of origin. Additionally, the continuous passage of these cells over the last 20-plus years has given rise to well-established differences in growth rate, hormone receptor status, karyotype and clonogenicity. Our purpose here was to characterize four primary dissociated tumor (DT) cell cultures from ER-negative breast tumors, the establishment of which we had previously described, to determine if they could provide more accurate models for both in vitro and in vivo studies. These four DT cultures (DT16, DT22, DT25 and DT28) display a triple negative phenotype and were characterized by their genomic profile by the PAM50 predictor analysis as belonging to the basal (DT22, DT25 and DT28) and luminal B (DT16) subtypes. Of those cultures for which primary tumor sample was available, microarray gene expression profiling and cell line clustering analysis show the primary tumor and dissociated culture cluster together and to similar breast tumors. Interestingly, of the three basal DT cultures, DT22 clusters with Claudin-low tumors and cell lines. We have established that each of these 4 DTs are tumorigenic in the NOD/SCID mouse model, and are now assessing their metastatic potential. DT16 generated metastases in 25% of the mice (6 out of 24; two in liver, four in lymph node). DT22 generated highly vascularized tumors, which may provide a relevant model for study of the in vivo vascular recruitment process. In addition, we have analyzed and characterized the role of pivotal signaling pathways often deregulated in breast cancer such as EGFR, P-MAPK, PI3k/mTOR, p53, TGFβ, Hedgehog, Notch, and Wnt in both the 2D culture as well as in sections of xenograft samples. Given that the scope of primary tumor heterogeneity and consequently the early stages of breast tumorigenesis are under-represented by current in vitro models in breast cancer research, the use of these DT cell cultures could more accurately recapitulate the disease in both the in vitro and in vivo settings.

P2-01-23
Seprase Promotes the Growth and Impairs the Migratory Ability of Breast Cancer Cells.
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Background: Seprase or Fibroblast Activation Protein alpha (FAP-a) is an integral membrane serine peptidase. It has been shown that it plays an important role in tumour proliferation, migration, invasion and angiogenesis. However, previous work has not satisfactorily explained both the suppression and promotion effects that have been observed. Furthermore, the interaction of Seprase with other membrane molecules or pathway had not been well investigated. This study aimed to investigate the role of Seprase in human breast cancer.

Methods: The full length of Seprase cDNA was isolated from normal human prostate tissue using RT-PCR and then cloned into an expression vector (pEF6-V5 His, TOPO). Following confirmation of successful cloning, the plasmid was transfected into the two human breast cancer cell lines, MCF-7 and MDA-MB-231. The function of Seprase in these two cell lines was then analysed using cell function assays and ECIS, and the potential interaction with other signalling pathways explored.

Results: Compared with the MCF-7 wild type cells (MCF-7 wt) and the MCF-7 cells transfected with pEF6/V5 empty vector (MCF-7 exp), the MCF7 cells transfected with Seprase (MCF-7 sef) had increased growth ability and impaired migratory ability. The growth rate of MCF-7 wt cells was 1132.72% on day 5 while those of MCF-7 exp and MCF-7 sef control cells were 897.24% and 990.99% respectively (p=0.011 and 0.032). The resistance increase of MCF-7 wt cells at 2 hours after wounding in ECIS assays was 478.22ohms compare with 1063.75 ohms and 1563.09 ohms in MCF-7 exp and MCF-7 exp cell lines (p<0.05). In MDA-MB-231 cells, over-expression of Seprase resulted in impaired motility. The resistance increase of the MDA-MB-231 wild type cells (MDA-MB-231 wt) and the MDA-MB-231 cells transfected with pEF6/V5 vector (MDA-MB-231 exp), and the MDA-MB-231 cells transfected with Seprase (MDA-MB-231 sef) at 3 hours after wounding in ECIS assay were 216.75ohms, 216.00ohms and 120.16ohms respectively. The growth of MDA-MB-231 cells and the adhesion and invasion ability of both MCF-7 cells and MDA-MB-231 cells were not dramatically influenced with Seprase expression. An inhibitor to focal adhesion kinase (FAK) restored the reduced motility ability of both MCF-7exp cells and MDA-MB-231exp cells. However, inhibitors to PI3K, ERK and ROCK had no influence on it.

Conclusion: These results suggest that Seprase promotes proliferation but inhibits the migration of breast cancer cells by potentially regulating the FAK pathway.
P2-01-24
Triple-Negative Breast Cancer Emerges from the Luminal Progenitor Compartement.

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Background: Triple-negative breast cancers (TNBC) are defined as tumors that lack expression of estrogen receptor (ER), progesterone receptor (PgR), and human epidermal growth factor receptor type 2 (HER2). Majority of TNBC (approximately 80%) are basal-like breast cancers. The question of whether there is a specific, identifiable cell in the normal breast form which basal-like breast cancers arise is controversial. We evaluated the frequency and receptor status of ductal carcinoma in situ (DCIS) and ductal components associated with T1 invasive ductal carcinoma (IDC) to clarify the developmental pathway of TNBC.

Methods: From our institutional archives between April 2000 and April 2011, we reviewed 758 cases of DCIS and T1 invasive ductal carcinoma without prior operative treatment. Immunohistochemistry for ER, PgR and HER2 was performed on ductal components and invasive components. Statistical analysis was performed using Fisher Extract Test.

Results: The frequencies of triple-negative (TN) subtype at each size were not seen significant differences. Ductal components were seen in 51 (84%) TN-IDC cases. Within 51 cases, the ductal components were TN in 49 cases. The remaining 3 cases were positive for ER and PgR, but negative HER2.

<table>
<thead>
<tr>
<th>Frequencies of each subtype</th>
<th>TN-HR+HER2-</th>
<th>TN-HR+HER2+</th>
<th>TN-HR-HER2+</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCIS</td>
<td>31 (7%)</td>
<td>115 (75%)</td>
<td>6 (4%)</td>
<td>21 (14%)</td>
</tr>
<tr>
<td>T1mic</td>
<td>2 (15%)</td>
<td>9 (59%)</td>
<td>1 (9%)</td>
<td>2 (13%)</td>
</tr>
<tr>
<td>T1a</td>
<td>18 (68%)</td>
<td>8 (28%)</td>
<td>1 (4%)</td>
<td>8 (14%)</td>
</tr>
<tr>
<td>T1b</td>
<td>8 (9%)</td>
<td>71 (79%)</td>
<td>14 (14%)</td>
<td>90</td>
</tr>
<tr>
<td>T1c</td>
<td>43 (9%)</td>
<td>329 (68%)</td>
<td>36 (8%)</td>
<td>35 (7%)</td>
</tr>
</tbody>
</table>

Frequencies of each subtype: TN-HR+HER2-: no expression of ER and/or PgR, but positive HER2; TN-HR+HER2+: positive ER and/or PgR, and positive HER2; TN-HR-HER2+: negative ER and/or PgR, and negative HER2. HR: hormone receptor

Conclusion: The resemblance between ductal components and invasive components and the coincident frequency of TNBC at each size sustain the hypothesis that TNBC arise from the luminal progenitor compartment.

P2-01-25
Truncated p110 ERBB2 (CTF611) Increases Migration and Invasion of Breast Epithelial Cells by Inhibiting STAT5b Activation.

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Background: Truncated ERBB2 receptors are present in a subset of human ERBB2+ amplified/overexpressing breast tumors, and are associated with trastuzumab resistance, metastasis, and poor clinical prognosis. However, whether truncated ERBB2 receptors are drivers of metastasis has not been well defined. In this study, we examined effects of full-length (p185) and truncated (p110) ERBB2 on the migration and invasion of human mammary epithelial cells, including HMLE and MCF10A cells.

Material and Methods: Recombinant p185 and p110 ERBB2 were stably expressed in human mammary epithelial cells (HMLE) and MCF10A cells via retrovirial vector. Expression of comparable levels of p185 and p110 in cells was confirmed by western blot. The phosphorylation states of downstream signaling proteins including STAT5 were assayed via phosphoimmunoblotting and Collaborative Enzyme Enhanced Reactive (CEER™) immunoassay. The effects of the p110 constructs on cell migration and invasion were investigated by transwell assays. shRNA-encoding lentivirus was used for specific silencing of STAT5b in HMLE cells, and STAT5b silencing was confirmed at the protein level using western blot.

Results and Discussion: Expression of p110 ERBB2 increased cell migration (HMLE, p = 0.04; MCF10A, p< 0.01) and invasion (HMLE, p= 0.03) when compared to expression of p185. Furthermore, expression of p110 in HMLE cells was associated with reduced phosphorylation of STAT5b. shRNA mediated silencing of STAT5b was sufficient to increase the migration (p < 0.01) and invasion of HMLE cells, phenocopying the p110 driven effects on HMLE cells. In clinical studies, loss of activated STAT5 protein correlates with breast cancer progression and is a negative predictor of survival. By analyzing publicly available gene expression datasets, we found that STAT5b mRNA expression is also significantly decreased in breast cancer compared to normal breast tissues in several studies, as well as in ERBB2 amplified vs. nonamplified samples. To our knowledge, this is the first reported perturbation of STAT signaling by truncated ERBB2 receptor, and suggests a mechanism by which truncated p110 ERBB2 (CTF611) increases migration and invasion of breast epithelial cells. This study extends the available data regarding STAT5 loss in breast cancer progression.

P2-01-26
TIMPv: a novel downstream transcriptional target gene that underpins CD146-suppressed breast tumor invasion.

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Background: CD146, a marker of endothelial cells, promotes tumor progression of many cancers including melanoma and prostate. Strikingly, several lines of evidence suggest that it is a suppressor of breast cancer (BC) progression. Not only the ligand(s) has not been identified, but CD146-downstream signaling mechanisms remain unknown.

Material and Methods: Here, we report the functional importance of CD146 and that of a novel transcriptional target of CD146-signaling, variant of tissue inhibitor of metalloproteinases (TIMPv) identified by microarray analysis, in underpinning the suppression of BC invasion, using novel validated Enhanced Green Fluorescent Protein (EGFP)-inducible systems of CD146 expression both in vitro and in vivo. Results: In functional experiments, induction of CD146 inhibited BC cell migration and invasion. TIMPv was identified by expression profiling as a novel transcriptional target of CD146-signaling, an association validated by quantitative PCR and immunoblotting experiments in a range of breast and melanoma cancer cells. However, siRNA inhibition of CD146 in the SKMel-28 melanoma cell line increased TIMPv expression, suggesting that while TIMPv is a positive transcriptional target of CD146 in BC cells, it is negatively regulated in melanoma cells. Furthermore, the functional relevance of TIMPv to CD146-suppressed metastasis was demonstrated by selective suppression of TIMPv in CD146-expressing BC inducible cells using RNAi. More interestingly, induction of CD146 expression in vivo using mouse EGFP-inducible system of CD146 expression resulted in suppression of breast tumor growth.

Discussion: Our study is the first report to provide a functional molecular link of TIMPv to cancer via unique axis that underpin TIMPv: a novel downstream transcriptional target gene that underpins CD146-suppressed breast tumor invasion.
P2-02-01  A Novel Inflammatory Breast Cancer-Specific Oncogene, Tazarotene-Induced Gene 1, Promotes Tumorigenicity and Invasiveness through the Receptor Tyrosine Kinase Axl.

Wang X, Sas0 H, Iwamoto T, Pusztai L, Gong Y, Woodward WA, Reuben JM, Hortobagyi GN, Ueno NT. Morgan Welch Inflammatory Breast Cancer Clinic; The University of Texas MD Anderson Cancer Center, Houston, TX; University of Texas MD Anderson Cancer Center, Houston, TX

Background: Inflammatory breast cancer (IBC) is the most lethal and aggressive form of breast cancer and is highly metastatic. The prognosis of patients with IBC is poor, and effective standard therapies for IBC are limited because the molecular mechanisms underlying the pathogenesis of IBC remain unknown. We recently found that tazarotene-induced gene 1 (TIG1) expression is significantly higher in IBC cell lines than in non-IBC cell lines. In both IBC and non-IBC data sets, estrogen receptor-negative/HER2-negative samples had significantly higher expression of TIG1 than did other clinical subtypes (estrogen receptor-positive/HER2-negative and HER2-positive). Therefore, we hypothesized that TIG1 plays an important role in the malignant process of IBC. In these studies, we determined the biological function of TIG1 in IBC cells and elucidated the molecular mechanism by which TIG1 regulates the invasiveness of IBC cells.

Methods: TIG1 expression in SUM149 and KPL-4 IBC cells was stably knocked down, and the effects of this knockdown on in vitro cell proliferation, migration, and invasion were analyzed. The effects of restoring TIG1 expression on TIG1-silencing IBC cells were also examined. To determine the tumorigenic activity of TIG1 in vivo, TIG1 stable-knockdown SUM149 cells and control shRNA-transfected cells were implanted into the mammary fat pads of athymic nude mice, and tumor growth was monitored. The receptor tyrosine kinase Axl, a potential functional partner of TIG1, was identified using DNA microarray analysis. The interaction between TIG1 and Axl in IBC cells was examined using immunoprecipitation and confocal microscopy assays. The signaling pathway in IBC cells in which TIG1 participates was also investigated.

Results: Knockdown of TIG1 expression in IBC cells reduced their proliferation, migration, and invasion in vitro. Also, silencing of TIG1 dramatically inhibited IBC tumor growth in a xenograft model. Moreover, restoring TIG1 expression rescued the proliferation, motility, and invasiveness of TIG1-silenced IBC cells. Most importantly, we identified Axl as a functional partner of TIG1 by showing that TIG1 interacted with and stabilized Axl in IBC cells. TIG1 regulated the invasiveness of IBC cells through mediation of the Axl signaling pathway. In SUM149 cells, TIG1 depletion decreased Axl expression, which led to downregulation of expression of matrix metalloproteinase-9, a molecule required for Axl-mediated invasion, and inactivation of nuclear factor-kB, ultimately leading to decreased invasiveness of IBC cells.

Conclusion: Our results identified TIG1 as an oncogenic gene that contributes to the tumorigenic and metastatic properties of IBC. Our data also linked TIG1 with the key tumorigenic gene Axl in IBC cells. Further studies designed to establish TIG1 as a therapeutic target in the treatment of patients with IBC are under way.

P2-02-02  Mutation of the APC Tumor Suppressor Stimulates Breast Cancer Cell Proliferation through Hyperactivation of FAK/Src/JNK Signaling.

Prosperi JR, Yang FF, Goss KH. University of Chicago, Chicago, IL

Background: The Adenomatous Polyposis Coli (APC) tumor suppressor gene is silenced by hypermethylation or mutated in as many as 70% of human sporadic breast cancers. In mouse models, germline mutation of APC disrupts normal mammary development and predisposes to mammary tumor formation. In an effort to more accurately model human breast cancer and determine the role for APC inactivation in the context of other oncogenic events, our laboratory has recently demonstrated that APC loss promotes mammary tumor development in the presence of the Polyoma virus middle T antigen (PyMT) oncogene, and results in a tumor phenotype resembling human metaplastic breast cancer. In this model, ApC mutation significantly enhances tumor cell proliferation but does not impact Wnt/β-catenin signaling. Because APC loss also induces mammary tumor cell spreading and morphological changes, we hypothesized that APC may regulate proliferation via signaling by the focal adhesion kinase (FAK) and Src (kinases) downstream of altered cell-matrix interactions.

Materials and Methods: Cell lines were established from primary cultures of experimental (MMTV-PyMT;ApCMin+/+) and control (MMTV-PyMT;ApCMin/-) mammary tumors and maintained in RPMI 1640 supplemented with 10% FBS and antibiotics at 37°C with 5% CO2. Cell proliferation was measured by incorporation of 5-bromo-2-deoxyuridine (BrdU), and subsequent immunofluorescence (IF) with an anti-BrdU antibody. Western blot analysis and IF were utilized to quantify the expression of functional signaling molecules and determine their localization, respectively. To investigate Wnt/β-catenin pathway activation, dual-luciferase reporter assays were performed using cells transfected with either Top-Flash or Fop-Flash reporter constructs.

Results: Cell lines isolated from mammary tumors from MMTV-PyMT;ApCMin/+ mice had altered morphology and increased rates of proliferation, both of which are consistent with our previous in vivo findings using this mouse model. In vitro, ApC mutation was associated with increased phosphorylation of FAK, Src and Jun kinase (JNK). Activation of both Src and JNK were required for the enhanced proliferation in the MMTV-PyMT;ApCMin/+ cell lines. In contrast, we did not observe any change in the expression of Wnt/β-catenin target genes, β-catenin subcellular localization, or β-catenin/TCF-mediated transcriptional activity. Combined, these data suggest that the proliferative and morphological changes mediated by APC loss in the PyMT breast cancer model are dependent on enhanced signaling downstream of cell-matrix interactions but do not involve the Wnt/β-catenin pathway.

Discussion: These data indicate that APC status is an important determinant of breast cancer cell behavior and specifically suggest that integrin-mediated signaling networks, including FAK, Src and JNK, are hyperactivated by APC loss to promote tumor cell proliferation. Moreover, these preclinical model systems offer a platform for further dissecting the molecular mechanisms by which APC contributes to breast carcinogenesis as well as identifying therapeutic strategies that may be effective in APC-deficient breast cancers.
P2-02-03
**MAP3K3, Amplified in Human Breast Cancer, Promotes Breast Tumor Progression and Defines Poor Prognosis.**

Dong J. Baylor College of Medicine, Houston, TX

Gene amplification in the 17q chromosomal region is observed frequently in breast cancers. An integrative bioinformatics analysis nominated MAP3K3 gene, located in 17q23, as a potential therapeutic target in breast cancer. This gene encodes the mitogen-activated protein kinase kinase kinase 3 (MEKK3), but has not yet been associated with cancer-causal genetic aberrations. We found that MAP3K3 was amplified in approximately 8-20% of breast carcinomas, and that its over-expression was an independent prognostic marker for poor outcome with respect to relapse-free and overall survival, especially among the estrogen receptor-positive breast cancer patients.

shRNA-mediated knockdown of MAP3K3 expression significantly inhibited cell proliferation and colony formation of MAP3K3-amplified breast cancer cell lines MCF7 and MDA-MB361, and promoted breast cancer cell apoptosis induced by TNFa, TRAIL, or a doxorubicin. In addition, ectopic expression of MAP3K3, in collaboration with Ras, induced colony formation in both primary mouse embryonic fibroblasts and immortalized mammary epithelial cells (MCF-10A). Together, these results suggest that MAP3K3 is a potential biomarker indicating poor prognosis, contributes to resistance to therapy, and is an oncogene in breast carcinogenesis. Therefore, therapeutic targeting of MAP3K3 may be attractive in breast cancer patients with MAP3K3-amplified breast cancer.

P2-02-04
**Novel Functions of vps34 in Non-Transformed Epithelial Cells: Regulation of Cell Proliferation and Tumorigenesis.**

Su H, Xu T, Ganapathy S, Yuan Z-M. UTHSCSA, San Antonio, TX

Autophagic and endocytic pathways are tightly regulated membrane rearrangement processes that are crucial for homeostasis, development and disease. A list of proteins regulating these cellular processes show altered expression in cancers, suggesting derailed autophagy or endocytosis as an emerging feature of cancer. Class III phosphatidylinositol 3-kinase (PI3kinase) Vps34 regulates both autophagic and endocytic systems by recruiting proteins containing PtdIns3P binding domains. Recently, many new insights into the biology of class III PI3Kinase have been reported. Most of these studies, however, used mainly transformed cells. It is unclear whether class III PI3Kinase works similarly in non-transformed cells. In the recent study, we explored this by depleting both transformed and non-transformed mammary epithelial cell lines of Vps34 using shRNA. In consistent with previous finding, vps34-depleted cells are defect in formation of FYVE-GFP puncta, and showed altered early and recycling endosome dynamics. In a shark contrast to transformed cells where knockdown of Vps34 was associated with decreased growth, Vps34 deficiency in non-transformed cells resulted in a marked increase in cell proliferation. The deregulated autophagy function found in Vps34-depleted cells appeared to be not involved in cell growth regulation as silence of either Atg7 or Beclin1 expression did not affect the growth rate. On the other hand, reporter luciferase-based ten-pathway screens suggested an altered activity of the MAPK pathway. Indeed, biochemical analysis indicated a sustained ERK activation resulting from the impediment of endosome-mediated EGFR turnover in Vps34 deficient cells. A contribution of the endosomal pathway to Vps34-mediated regulation of cell proliferation was further confirmed by using Rab5 and Rab11 mutants that deregulated early and recycling endosome, respectively. Importantly, inactivation of Vps34 compromized the cellular barrier against oncogene-mediated transformation, as demonstrated by the increased susceptibility of Vps34-depleted cells to Ras-induced colony formation in vitro and tumor development in mice. All together, our data uncover a novel role for the class III PI3Kinase-endosomal pathway in regulation of cell proliferation.

P2-02-05
**Molecular Mechanism for Src Homology Phosphotyrosyl Phosphatase 2 Regulation of Cell Motility and Migration.**

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Background: The Src homology phosphotyrosyl phosphatase 2 (SHP2) acts as a transducer of mitogenic and cell survival signaling downstream of receptor tyrosine kinases, cytokine receptors and integrins. As such, SHP2 promotes cell growth, transformation, survival, and motility, among other processes. Recent studies suggest that SHP2 is overexpressed in breast cancer and is essential for the maintenance of the transformed phenotype both in the HER2-positive and the basal-type/triple-negative breast cancer (BTBC) cells.

Materials and Methods: The expression of SHP2 in BTBC cells was suppressed constitutively by lentivirus-mediated transduction of specific shRNA. Impact of SHP2 inhibition on cell migration was determined by the monolayer wound healing assay. The SHP2 substrate-trapping mutant was used to identify FAK as an SHP2 substrate. Site-directed mutagenesis, binding studies and phosphatase assays were employed to further characterize FAK as a bona fide SHP2 substrate. Immunofluorescence microscopy was conducted to visualize the amounts of phosphorylated FAK and its location in the cell after adhesion.

Results: We demonstrate that SHP2 is essential for the migration of BTBC cells as evidenced by loss of the enhanced migratory behavior upon its inhibition. Using the SHP2 substrate-trapping mutant, FAK was identified as a biological substrate of SHP2. Site-directed mutagenesis, binding studies and phosphatase assays confirmed that SHP2 indeed associates with and dephosphorylates FAK at the pY397 site, a site known to promote the focal adhesion activity of FAK. Immunofluorescence of breast cancer cells with SHP2 inhibition were found to have higher levels of FAK pY397 further confirming that FAK is an SHP2 substrate. The increase in pY397 level correlated with loss of cell polarity, enhanced focal adhesion formation, and acquisition of non-transformed morphology.

Discussion: These results suggest that dephosphorylation of pY397 of FAK by SHP2 is the major mechanism by which it is able to modulate cancer cell migration. Together with previous findings, the current studies show that SHP2 is a suitable target for therapeutic intervention to combat breast cancer metastasis.

P2-02-06
**Inhibition of CDK4 Phosphorylation of Smad3 Decreases Cyclin D Overexpressing Breast Cancer Cell Proliferation and Migration.**

Tarasewicz E, Hardy A, Straehla J, Foucar C, Zelivianski S, Jeruss J. Northwestern University Feinberg School of Medicine, Chicago, IL

Introduction: Several aspects of breast cancer onset and disease progression have been linked to members of the TGFβ superfamily and their associated downstream signaling components, the Smads. Alterations in Smad signaling have been directly implicated in the dichotomous role of TGFβ in malignancy, enacting both tumor suppressant and tumor promoting behaviors in breast carcinogenesis. Our previous work characterized Smad3 as a tumor suppressor and found that Smad3 action is inhibited upon phosphorylation
by cyclin D/CDK4 in breast cancer cells. Others have shown that CDK phosphorylation of Smad3 can affect cell motility. Based on these findings, we hypothesized that activation of CDK4 leads to phosphorylation and inhibition of Smad3, thus releasing cell cycle arrest and promoting cell proliferation and metastasis.

Methods: MCF7 parental and cyclin D overexpressing cells (MCF7 CD1) were treated with a CDK4 inhibitor alone or in combination with doxorubicin or paclitaxel and cell proliferation was determined using the MTS assay. MCF7 study cells were also transduced with lentiviral empty vector, wild-type (WT) Smad3, or Smad3 containing mutant CDK sites (T179V and 5M) resistant to inhibitory cyclin D/CDK phosphorylation. Stably transduced cells were plated in Matrigel 3-dimensional (3D) culture and treated with CDK4 inhibitor alone or with doxorubicin over a 12 day time course. Colony area was measured using MetaMorph software. Transwell migration and Matrigel invasion assays were used to determine the effect that transfection with Smad3 CDK phosphorylation site mutants or treatment with a CDK4 inhibitor had on highly metastatic cyclin D expressing MDA-MB-231 cells. Study wells were fixed/stained and the number of migrated or invaded cells was counted.

Results: Treatment with the CDK4 inhibitor alone resulted in a growth inhibitory effect on proliferation of MCF7 parental and CD1 cells. Treatment with the CDK4 inhibitor in combination with chemotherapy resulted in the greatest inhibition of cell proliferation. In 3D culture, colonies formed by MCF7 study cells transduced with T179V or 5M Smad3 mutant constructs had smaller acinar size when compared with vector-transduced control cells. When compared to untreated cells, the MCF7 CD1 cells treated with CDK4 inhibitor or chemotherapy alone had smaller acinar size, with greatest decrease in size observed when the treatments were combined. Lastly, MDA-MB-231 cells transfected with the 5M Smad3 mutant construct, containing mutations in all 5 CDK phosphorylation sites, resulted in the greatest decrease in cell migration and invasion, when compared with cells transfected with both vector control or WT Smad3. Treatment of the MDA-MB-231 cells with the CDK4 inhibitor also resulted in a significant decrease in cell migration and invasion.

Conclusions: Mutation of CDK phosphorylation sites in the Smad3 construct or direct inhibition of CDK4 resulted in a decrease in breast cancer cell proliferation, 3D colony formation and cell migration and invasion. Treatment with the CDK4 inhibitor in combination with chemotherapy further augmented these results. Inhibition of CDK4 and restoration of Smad3 activity may have a role in the treatment of breast cancers overexpressing cyclin D.

P2-02-07
Regulation of mTOR Signaling by Proto-Oncogene PELP1.
Gonugunta VK, Cortez V, Rethman C, Nair BC, Sareddy GR, Vadlamudi RK. UTHSCSA, San Antonio, TX

BACKGROUND: Despite positive effects of hormonal therapy, initial or acquired resistance to endocrine therapies frequently occurs. Emerging evidence suggests that ER activity is complex and requires functional interactions with coregulators. In addition, ER also participates in extra-nuclear signaling events in the cytoplasm and growth factor cross talk with ER is implicated in the development of therapy resistance. The mammalian target of rapamycin (mTOR) is a serine/threonine kinase that plays an important role on cell growth and proliferation. Proline, Glutamic-acid and Leucine-rich Protein 1 (PELP1) is an ER coregulator that functions in nuclear as well as extra-nuclear actions. PELP1 functions as a proto-oncogene and is a prognostic indicator of shorter breast cancer specific survival. The objective if this study is to examine whether PELP1 participates in ER-growth factor signaling cross talk via mTOR pathway.

METHODS: To test this hypothesis, we have used ER-positive model cells (MCF7, ZR75) along with PELP1 over expressing (MCF7-PELP1, ZR75-PELP1) and PELP1 knock down cells (MCF7-PELP1 shRNA, ZR-75-PELP1 shRNA). Estrogen and Heregulin were used as signal inducers. Rapamycin that inhibit mTORC1 and AZD8055, an inhibitor of both mTORC1 and mTORC2 activities were used as pharmacological inhibitors to block mTOR pathway. The affect of blocking mTOR axis on PELP1 mediated oncogenic functions was determined using ERE and E2F reporter gene assays, cell proliferation (MTT) and anchorage independent assays. Cell cycle progression was monitored by flow cytometry. PELP1 modulation of mTOR signaling was validated using siRNA approach in MCF7, ZR75 cells and/or by PELP1 overexpression in PELP1-shRNA clones and evaluated the role of identified components for changes in the mTOR signaling using Western analysis. IHC analysis of mTOR signaling components was performed using tissues collected from PELP1 overexpressing and PELP1 siRNA lenti homoe treated tumors.

RESULTS: Over expression of PELP1 enhanced the estrogen and heregulin mediated cell proliferation in breast cancer cells. Knock down of PELP1 with siRNA significantly reduced the activation of mTOR signaling components (p70S6K and 4E-BP1). Accordingly, over expression of PELP1 in the breast cancer cells correlated with increased mTOR signaling. Pharmacological inhibition of mTOR substantially reduced PELP1 mediated ER coactivation functions in ERE reporter gene assays. Rapamycin (10^-5M) or AZD8055 (10^-M) abolished PELP1 mediated growth advantage in estrogen and heregulin stimulated breast cancer cells. Further, combinatorial treatment of mTOR inhibitors sensitized PELP1 overexpressing cells to tamoxifen therapy. IHC studies using xenograft tissues with PELP1 overexpression or down regulation, showed correlation of PELP1 expression with the activation of mTOR signaling components.

CONCLUSIONS: Our results suggest that PELP1 driven oncogenic functions involve PELP1 modulation of mTOR signaling and blockage of mTOR signaling render PELP1 driven tumors highly sensitive to therapeutic inhibition.

P2-02-08
Int6 Regulates Both Protesomal Degradation and Translation Initiation and Is Critical for Proper Formation of Acini by Human Mammary Epithelium.
Seo J, Snider SJ, Lloyd RE, Chang EC. BCM

The mammalian INT6/EIF3E gene has been implicated in breast tumor formation, but its functional activities remain poorly defined. Consistent with the fact that Int6 is frequently down-regulated in human breast tumors, we found that repressing INT6 expression induced transformed properties in normal human mammary epithelial cells (MCF10A) in both 2D and 3D cultures. Int6 contains a PCI domain, found in several components of both the proteasome and the translation initiation factor eIF3, suggesting that it can functionally interact with both. Indeed, our data show that Int6 associates with proteasomes in human cells, and that INT6 knockdown prevents proper assembly of active proteasomes. In addition, we show that down-regulation of INT6 reduces translation initiation (both polysome formation and global protein synthesis). However, Int6 regulates translation selectively, reducing translation from cap-dependent and Becl-2- IRES reporters, but stimulating translation from the CVB3 IRES. These data collectively suggest that Int6 controls both protein synthesis and protein degradation, and is thus capable of fine-tuning
protein levels. Abnormal levels of regulatory proteins caused by Int6 abnormalities may thus be responsible for disrupting normal morphogenesis of mammary epithelial cells and causing transformed phenotypes.

P2-02-09
TP53 Mutation Patterns in Breast Cancer Subgroups.

Tumor protein 53 (TP53) is the most commonly altered gene in human cancers. In breast cancers, TP53 is mutated in approximately 30% of all cases, but this frequency fluctuates widely within the different molecular subclades. Different types of mutations may be observed, such as substitutions (replacement of a nucleotide pair by another one), or complex mutations (deletions or insertions of one or more nucleotides). Mutation types may reflect mechanism of DNA lesion or DNA repair deficiencies. Furthermore, mutations can give rise to different effects such as truncating mutations leading to loss of function, or missense mutations often leading to dominant negative activity. Those mutation effects can be advantageous in tumorigenesis and thus can be subject to selective pressure.

Here we classified 572 breast tumors in three groups, according to microarray data: luminal, basal and molecular apocrine. TP53 status was assessed by a yeast-based functional assay (FASAY) and cDNA sequencing. We then assessed whether any feature of TP53 mutations would be preferentially associated to a specific subtype of breast cancer.

- In term of TP53 mutation frequencies, as expected, lowest frequency was observed in luminal subgroup (26%) and highest in basal (90%) and molecular apocrine (70%). Notably, much higher rate of TP53 mutations occurred in luminal B subgroup (41%) than in luminal A one (17%), suggesting that TP53 may be an important feature in progression from luminal A to B.

- In term of mutations types, luminal tumors showed high frequency of substitutions, while molecular apocrine and basal presented increased rate of deletions and insertions, reflecting probably increased rate of DNA breaks. This suggests that same mutational events may occur in basal and molecular apocrine tumors.

- In term of mutation effects, we found high frequency of missense mutations in luminal tumors (notably AT to GC) and much higher rate of truncating mutations in basal tumors. These observations point to an existence of different selection pressure in each of them, such as a strong pressure for P53 mutations with potential dominant negative inhibition of P73/P63 (recently shown to favor invasion), in luminal tumors. Collectively, these results point not only to different mechanisms of P53 gene inactivation, but also different functional consequences among the different breast cancer subclades.

P2-03-01
Genetic Reduction of Circulating Insulin-Like Growth Factor (IGF)-1 Differentially Impacts the Effects of Diet-Induced Obesity and Calorie Restriction on Mammary Tumor Progression.
Ford NA, Nunez NP, Perkins SN, Hursting SD. University of Texas at Austin, Austin, TX; National Cancer Institution, Rockville, MD

Background: Epidemiological evidence suggests that obesity worsens breast cancer prognosis in pre- and postmenopausal women. Previously we demonstrated that diet-induced obese (DIO) mice and rats have higher circulating levels of IGF-1 and enhanced mammary tumor burden compared to normoweight controls. In contrast, we have found in several mouse models that a 30% calorie restricted (CR) diet regimen reduced serum IGF-1 levels and decreased mammary tumor growth. The present study investigated the effects of CR and DIO on mammary tumor growth in mice with normal serum levels of IGF-1 (wild-type) or mice with reduced circulating levels of IGF-1 (LID).

Our goal was to identify new targets and strategies (with a focus on the IGF-1 pathway) for breaking the obesity-breast cancer link, which is important given the high prevalence of obesity in the U.S., and the strong association between obesity and breast cancer.

Materials and Methods: Virgin female liver-specific IGF-1-deficient (LID) and wild-type (FVB/Ncr) mice (10-12 weeks of age) were randomized (n=10-14 per group) to receive: control diet (modified AIN-76A), a 30% CR regimen, or a DIO regimen (60 kcal% fat diet). Mice consumed the experimental diets for 11 to 15 weeks prior to orthotopic injection (4th mammary fat pad) of Met-1 tumor cells derived from a polyoma middle-T antigen transgenic mouse mammary tumor. Transplanted tumors were allowed to grow for 4 weeks and tumor volume was measured by electronic calipers biweekly. Mice were then killed, serum was collected and
stored at -80°C, and tumors were excised, weighed and either formalin-fixed, paraffin-embedded or flash-frozen.

Results: Using the LID mouse model of IGF-1 deficiency, this study established that decreased circulating IGF-1 (relative to wild-type mice) dramatically reduces Met-1\(^{fvb2}\) mammary tumor growth regardless of diet treatment (P=0.034). We also showed in LID mice that CR (44 +/- 15.6 mm\(^3\)), relative to control diet (89 +/- 33 mm\(^3\)) has no significant effect on Met-1\(^{fvb2}\) tumor growth, suggesting nearly all of the suppressive effects of CR on tumor growth (at least in the Met-1\(^{fvb2}\) model), can be explained by reduced systemic IGF-1. In contrast, while most of the DIO effect on tumor growth was ablated in the LID mice (332 +/- 104 mm\(^3\)) vs. wild-type mice (3130 +/- 421 mm\(^3\)), tumor growth in LID/DIO mice was significantly higher than LID/control mice (89 +/- 33, P=0.006) despite no change in IGF-1 levels, suggesting that a mechanism independent of IGF-1 contributes to some of the effects of DIO on Met-1\(^{fvb2}\) mammary tumor growth. Within LID mice, serum insulin, resistin and adiponectin were not modified by the CR or DIO diet.

Discussion: We conclude that components of the IGF-1 pathway represent promising mechanistic targets for mimicking the anti-cancer effects of CR and breaking the obesity-breast cancer link. However, there are likely other mechanisms independent of IGF-1 that contribute to the breast tumor enhancing effects of DIO and that need to be defined.

P2-03-02
An IGF1R Antibody Does Not Inhibit Growth of Tamoxifen Resistant MCF-7 Cells.
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The role of the insulin-like growth factor (IGF) system in breast cancer has been well defined, and inhibitors of this pathway are currently in clinical trials. The majority of anti-IGF1R clinical trials are in estrogen receptor-positive patients who have progressed on prior endocrine therapy with some early reports showing no benefit for addition of IGF1R inhibitors to endocrine therapy. The effect in endocrine-resistant models, mimicking the clinical trial scenario, has not been adequately investigated. To examine the effectiveness of IGF1R inhibitors in vitro, tamoxifen-resistant (TamR) cells were generated by culturing MCF-7L cells in the presence of 4-hydroxy-tamoxifen for more than 1 year. TamR cells had diminished levels of IGF1R with unchanged levels of insulin receptor (IR), and enhanced phosphorylation of Akt. Further, TamR cells failed to further respond to IGF-1-induced Akt activation while retaining responsiveness to both insulin and IGF-II. Additionally, IGF-1 failed to enhance the proliferation and anchorage-independent growth of TamR cells; however, both insulin and IGF-II stimulated proliferation and anchorage-independent growth. An IGF1R antibody (dalotuzumab) was able to inhibit IGF-I-mediated Akt phosphorylation, proliferation, and anchorage-independent growth in parental MCF-7L cells and had minimal effect on insulin and IGF-II stimulation. In TamR cells, dalotuzumab failed to inhibit proliferation or anchorage-independent growth in response to either insulin or IGF-II. An IGF1R tyrosine kinase inhibitor, (AEW 541) with equal potency for the IGF1R and IR, inhibited IGF-I, IGF-II, and insulin-stimulated Akt phosphorylation, proliferation, and anchorage-independent growth in parental MCF-7L cells. Interestingly, AEW 541 also inhibited insulin- and IGF-II-stimulated Akt phosphorylation, proliferation, and anchorage-independent growth in MCF-7L TamR cells. We conclude that cells selected for tamoxifen resistance in vitro have downregulated IGF1R making antibodies directed against this receptor ineffective. Preliminary studies in vivo, suggest that initial co-treatment with tamoxifen plus dalotuzumab was more effective than either drug alone. In contrast, acquired tamoxifen resistance might require dual inhibition of IGF1R and PI3K targets to completely suppress IGF system signaling as currently under study in the clinic. Alternatively, IGF1R tyrosine kinase inhibitors may be effective by inhibiting both IGF1R and IR signaling in cells selected for tamoxifen resistance.

P2-03-03
An Insulin-Like Growth Factor I (IGF-I)-Induced Gene, Solute Carrier Family 7 Member 11 (SLC7A11)/xCT, Mediates IGF-I-Induced Biological Behaviors in Breast Cancer Cells.
Yang Y, Becker MA, Yee D. University of Minnesota, Minneapolis, MN

Our laboratory studied the gene expression profiles of a series of T47D variant cell lines with differential insulin receptor subtype (IRS) adaptor protein expression to develop predictive IGF-I pathway biomarkers. We identified an IGF-I-induced gene, SLC7A11 (or xCT), which is specifically regulated through IRS-1. xCT encodes the cystine/glutamate transporter subunit of the heterodimeric amino acid transport system xc- which is a major plasma membrane transporter for the cellular uptake of cystine in exchange for intracellular glutamate. xCT is involved in the regulations of proliferation, metastasis, and drug resistance in various cancers. However, to date, the linkage between xCT and the IGF-I signaling pathway has not been described. To study the role for xCT in mediating IGF-I-induced biology in breast cancer cell lines, we examined xCT mRNA expression upon IGF-I stimulation in two breast cancer cell lines; the MCF-7 (IRS-1 activated) and MDA-MB-231 (IRS-2 activated) cells. Significant increased xCT expression was observed only in MCF-7 cells after IGF-I treatment. Immunoblots showed that xCT protein expression was elevated after IGF-I treatment and induced glutamate/cystine exchange in MCF-7 cells. shRNA was used to downregulate xCT in MCF-7 and MDA-MB-231 cells. In MCF-7, IGF-I-stimulated cell monolayer growth was suppressed by xCT shRNA or by the xCT inhibitor sulfasalazine (SASP). In MDA-MB-231 cells, xCT downregulation did not affect IGF-mediated Boyden chamber migration. Thus, IGF-I induction of cellular xCT levels is associated with cell growth in the IRS-1 activated MCF-7 cells, while MDA-MB-231 cells were not affected by downregulation of this gene. Therefore, our data imply that xCT may mediate IGF-I induced biological functions in breast cancer cell lines through an IRS-1 dependent pathway.

P2-03-04
Novel Pathways Underlying the Initiation and Transition of DCIS to IDC of HER2-Overexpressing Breast Cancer Model.
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Background: The HER2/Neu -oncogene is amplified in 20-25% of human primary breast cancers and this alteration is associated with disease behaviour. The HER2/neu oncogene encodes a receptor-like tyrosine kinase, whose overexpression in breast cancer predicts poor prognosis and resistance to conventional therapies. Whereas signalling pathways emanating from HER2 have been characterized, much less is known about the transcriptionally regulated genes controlled by HER2 that contribute to the initiation of Ductal Carcinoma Insitu (DCIS) and their transition to an invasive ductal carcinoma (IDC).
Materials and Methods: Normal and HER2 overexpressing mammary epithelial cells (MCF10A) were grown in extracellular matrix to form 3D structures. When grown in extracellular matrix as 3-dimensional spheroids, control cells developed a hollow lumen, but HER2-overexpressing cells populated the lumen by evading apoptosis. On the next step, upon the growth factor stimulation HER2-overexpressing cells grown in extracellular matrix, which protruded invasive outgrowths. Highly variable genes were selected from the described phenotypes using RNA isolated from the 3D structures and hybridized to an Affymetrix HuGene 1.0 ST oligonucleotide array.

Results: Using microarrays we analysed transcriptional events responsible for the morphological changes and found several novel sets of genes such as integral proteins, transcription factors, matrix proteases, Notch genes and chemokines were highly altered in the HER2 overexpressing group which were not appreciated before. Using gene annotation we defined molecular-pathways responsible for the phenotypical changes. More specifically, our study proposes a two hit model describes the pathways involved in the initiation of the Ductal Carcinoma In situ and their transition to Invasive Cancer underlying the HER2 transcriptional network.

Discussion: According to the proposed model, expansion of ductal hyperplasia is limited by intraluminal apoptosis, unless they overexpress HER2, which drives proliferation and forms DCIS due to HER2 induced Notch pathways genes. Secondly, results obtained with the 3D system and their reflection in clinical outcome, we propose that neither HER2 amplification nor the presence of GFs, is sufficient for development of IDC, but their co-occurrence can instigate metastasis by the activation of genes of integrin-adhesion signaling. The more virulent scenario combines HER2 amplification with GFs, thereby switching a robust, auto-stimulatory program.

P2-03-05
Lin S, Yang J, Ellekholou AG, Bandyopadhyay A, Wang L, Cornell JE, Yeh F-T, Aygijn JK, Sun L-Z. University of Texas Health Science Center; San Antonio, TX; NIHGRF-NIH, Bethesda, MD

Triple negative, basal-like breast cancer is highly aggressive and has a poor prognosis. The molecular mechanisms that drive its progression are elusive and no molecular target has been identified for its prevention or treatment. Here, we demonstrate that triple negative human breast cancer cells and clinical samples show an attenuated transforming growth factor-beta (TGF-β) signaling. Therefore, we developed a series of isogenic basal-like human mammary epithelial cells (HMECs) with altered TGF-β sensitivity and different malignancy, resembling a full spectrum of basal-like breast carcinogenesis, and determined the molecular mechanisms that contribute to oncogene-induced transformation of basal-like HMECs when TGF-β signaling is attenuated. We found that expression of a dominant-negative RI1 (DNRII) receptor of TGF-β1 abrogated autocrine TGF-β1 signaling in telomerase-immortalized HMECs and suppressed H-ras-V12-induced senescence-like growth arrest (SLGA). Furthermore, co-expression of DNRII and H-ras-V12 rendered HMECs to become highly tumorigenic and metastatic in vivo in comparison with H-ras-V12-transformed HMECs that spontaneously escaped H-ras-V12-induced SLGA. By using microarray analysis, we found that p21 is the major player mediating Ras-induced SLGA and attenuated or loss of p21 expression contributed to the escape from SLGA when autocrine TGF-β signaling is blocked in HMECs. Furthermore, knockdown of p21 also suppressed H-ras-V12-induced SLGA. Our results identifies that autocrine TGF-β1 signaling is an integral part of cellular anti-transformation network by suppressing the expression of a host of genes including p21-regulated genes that mediate oncogene-induced transformation in basal-like breast cancer.

P2-03-06
Endothelin-1/Endothelin A Receptor Signalling in Breast Cancer.
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Background: Endothelin-1 (ET-1) and endothelin A receptor (ETAR) are implicated in breast cancer growth and progression. ET-1 is secreted by both tumor cells and stroma (macrophages, endothelial cells). The purpose of this study is to evaluate ET-1/ETAR role on cell proliferation and its effects on EGFR signaling pathway.

Materials and Methods: Two breast cancer cell lines: MCF-7 and MDA-MB-231 were stimulated with ET-1. Proliferation of breast cancer cells was analyzed using MTT assay. Protein expression (EGFR, pEGFR, AKT, pAKT, ERK, pERK ) was evaluated by Western blot. siRNA knockdown of endothelin A receptor (ETAR) was performed in MCF-7 and MDA-MB-231.

Results: ET-1 stimulated proliferation in both cell lines. Interestingly, at higher ET-1 concentrations cell proliferation was more pronounced in MCF-7 when compared with MDA-MB-231. Stimulation with ET-1 activated EGFR and downstream signaling proteins (pAKT and pERK) in both cell lines. Incubation of breast cancer cells with ET-1 for 48 hours had different effect on total EGFR level: 3 fold increase of total EGFR was seen in MCF-7, while no change was detected in MDA-MB-231. siRNA against ETAR decreased ET-1 induced cell proliferation and reduced total EGFR level in MCF-7.

Conclusions: These observations suggest that ET-1/ETAR plays an important role in survival and proliferation of breast cancer cells. However, those effects are diverse in different subtypes of breast cancer. Our results may represent an improved selective targeted treatment strategy, especially for estrogen receptor positive breast cancer.

P2-04-01
Extracellular PAI-1 – t-PA – IGFBP3 Cascade Mediates Chemotherapy-Induced Senescence of Breast Cancer Cells.
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Upon successful chemotherapy, cancer cells undergo either apoptosis or senescence. Whereas the mechanism of cancer cell apoptosis is relatively well understood, there is only scant knowledge on how chemotherapy-induced cancer senescence occurs. Here we report that a cascade of secreted proteins, plasminogen activator inhibitor 1 (PAI-1) - tissue-type plasminogen activator inhibitor (t-PA) – insulin-like growth factor-binding protein 3 (IGFBP3), mediates chemotherapy-induced senescence of breast cancer cells.

MCF-7 breast cancer cells display robust senescence induction upon doxorubicin treatment and we found that the conditioned medium from senescent MCF-7 cells can induce senescence in non-senescent MCF-7 cells, which suggested the presence of secreted mediator(s) of senescence. To identify such mediator(s), we undertook a quantitative proteomic analysis of the protein secretion from senescent MCF-7 cells and observed significantly increased levels of secreted IGFBP3. Increased extracellular IGFBP3 levels were also observed upon doxorubicin-induced senescence of ZR75-1 breast cancer cells and primary mammary epithelial cells. We demonstrated that IGFBP3 induces senescence in breast cancer cells, which requires the Rb and
p53 pathways. Conversely, shRNA-mediated knock-down of IGFBP3 alleviated doxorubicin-induced senescence of breast cancer cells. These results suggest that IGFBP3 functions as a secreted mediator of chemotherapy-induced breast cancer senescence.

To gain insight into the regulation of IGFBP3-induced senescence, we undertook a proteomic screening for IGFBP3 interactors in the secretome and identified t-PA as candidate interactor. t-PA is a secreted protease that is known to cleave and activate plasminogen. We found that t-PA cleaves IGFBP3 and abolishes IGFBP3-induced or doxorubicin-induced senescence of breast cancer cells. The protease activity of t-PA toward plasminogen is inhibited by PAI-1 and we demonstrated that PAI-1 also protects IGFBP3 from cleavage by t-PA. Interestingly, PAI-1 was previously identified as an inducer of senescence that acts downstream of p53 (Nature Cell Biology 8:877-84, 2006). We showed that IGFBP3 knock-down by shRNAs abolishes senescence induction by PAI-1, suggesting that IGFBP3 is a critical downstream mediator of PAI-1-induced senescence. We also observed dramatically increased extracellular PAI-1 levels upon doxorubicin-induced senescence of breast cancer cells. Importantly, RNAi suppression of PAI-1 in breast cancer cells resulted in concomitant suppression of extracellular IGFBP3 levels upon doxorubicin treatment and abrogation of senescence induction. Taken together, these results suggest a role for extracellular PAI-1 – t-PA – IGFBP3 cascade in mediating chemotherapy-induced senescence of breast cancer cells.

This study uncovered the extracellular components of senescence signaling for chemotherapy-treated breast cancer cells. Senescence mediators secreted from chemotherapy-treated breast cancer cells may amplify the senescence response and provide a non-cell autonomous tumor suppression mechanism. These extracellular senescence mediators could be exploited to increase the efficacy of breast cancer chemotherapy.

**P2-04-02**

Identification of a Novel Glycosyltransferase-Like Gene as an Autophagic Inducer in Human Mammary Carcinoma Cells Via Down Regulation of BCL-2.

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Here we report the identification of a previously undescribed glycosyltransferase-like gene, GLTx, and its cellular effect on mammary carcinoma cells. GLTx codes for a novel human protein which is highly conserved in primates. Data obtained from the mRNA expression database Oncomine, demonstrate that GLTx is differentially expressed in a variety of cancers including breast cancer. Western blot analysis of a multiple human tissue blot using a rabbit polyclonal antibody against GLTx demonstrated that GLTx was highly expressed in liver, moderately in kidney, intestine and stomach and undetectable in any other tissues. To functionally characterize GLTx, we attempted to establish stable cell lines with forced expression. However, forced expression of GLTx was completely lethal and resulted in severe cell death in the mammary carcinoma cell lines, MCF-7 and BT549, as well as in any other cell lines tested. Staining of MCF-7 cells transiently transfected with a GLTx expression plasmid by either Hoechst 33258 or fluorescein isothiocyanate-conjugated annexin V and propidium iodide confirmed that the observed cell death was not caused by apoptosis. Further investigation confirmed that GLTx expressing cells exhibited cytolytic accumulation of autophagosomes, consistent with the induction of autophagy, as measured by the fluorescently labelled autophagosome marker LC3. In addition we have shown that GLTx downregulated BCL-2, an inhibitor of autophagy, as measured by BCL-2 gene promoter luciferase activity assay and western blot assay following transient transfection of a GLTx expressing plasmid. Thus, we have demonstrated that GLTx is a novel autophagic inducer that causes cell death by down regulating BCL-2.

**P2-05-01**

Acquired Tamoxifen Resistance Promotes Angiogenic Responses in ER+ Breast Cancer Cells.

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Background: In the treatment of pre-menopausal women with oestrogen positive (ER+) breast cancer, tamoxifen represents a first line of adjuvant treatment with demonstrable benefits. Despite this, resistance is frequently acquired to tamoxifen with an associated poor prognosis. Breast cancer cell models have revealed the importance of growth factor signalling networks in sustaining growth of endocrine-resistant cancers and, more recently, their ability to promote a highly migratory and invasive phenotype, together with the expression of genes with pro-angiogenic ontology. The potential of endocrine resistant cells to elicit angiogenic responses, however, remains unknown.

Materials and Methods: Real-time PCR was used to validate results from preliminary Affymetrix-based gene profiling of pro-angiogenic gene expression in endocrine-sensitive MCF7 cells and their tamoxifen-resistant (TamR) counterparts. The expression of pro-angiogenic factors in conditioned media (CM) from these cells was assessed by ELISA. The proliferative and migratory effects of MCF7 and TamR CM on vascular endothelial cells (HUVEC and HECV cells), was determined by MTS cell proliferation assay, wound closure assays and Matrigel tube formation assays. Changes in endothelial cell migration following co-culture with endocrine-resistant cells were examined using Boyden-chamber chemotaxis assays. Growth factor signalling and migration pathway activation in endothelial cells in response to CM was determined by Western blotting.

Results: TamR cells were found to express high levels of HIF-1α, IL-8 and VEGF-A at an mRNA level compared with expression in MCF7 cells. High levels of VEGF-A protein were also confirmed in the conditioned media from TamR cells versus their endocrine-sensitive counterparts. TamR conditioned media promoted endothelial cell proliferation, migration and the formation of tubules to a greater extent than that seen in MCF7 CM treated cells. TamR conditioned media was found to stimulate VEGFR2 phosphorylation and downstream activation of MAPK in endothelial cells compared to MCF7 CM. Pharmacological inhibition of VEGFR2 activity in endothelial cells suppressed TamR-induced endothelial cell proliferation and VEGFR phosphorylation. Further pharmacological manipulation of erbB receptors and intracellular kinases in TamR cells revealed an ERGF/Her2-Src kinase dependent mechanism of VEGF-A production in these cells.

Discussion: These data suggest acquired tamoxifen resistance is accompanied by development of an erbB receptor/Src kinase-dependant pro-angiogenic phenotype which, if recapitulated in vivo, may promote tumour progression. Therapeutic targeting of erbB/Src axis may prove beneficial in such cases.
P2-05-02

PGC1, Peroxisome Proliferator Activated Receptor-gamma (PPAR-gamma) Coactivator-1, Is Necessary in PPAR-gamma Modulated Angiogenesis.
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Background. Peroxisome proliferator activated receptor-gamma (PPAR-gamma), a nuclear hormone receptor, has been shown to be an important regulator of gene expression. PPAR-gamma agonists have been shown to have anti-cancer properties by ways of inducing expression of tumour suppressor genes. It has been indicated that this action of PPAR-gamma is manifested by forming a complex with the PPAR-gamma coactivators (PGC1 & 2) and in association with other transcription factors such as SRC-1 and CREB binding proteins. Both PPAR-gamma and PGC-1 have been shown to be aberrantly expressed in human cancer including human breast cancer (1). PPAR-gamma also plays a role in regulating angiogenesis and that PPAR-gamma agonists have been shown to have anti-angiogenic functions. In the present study, we investigated the role of PGC-1 in PPAR-gamma mediated actions on vascular endothelial cells.

Materials and methods. Mammalian expression systems for human full length PGC-1 were constructed again using human mammalian expression vector in pCDNA3.1. Anti-PGC-1 transgenes were constructed again using human mammalian expression vector in order to knock down PGC-1 transcript from the cells. Human vascular endothelial cell, HECV, was used in the study. Cell functions linked to angiogenesis including cell adhesion and cellular migration was evaluated using the electric cell impedance sensing method. PPAR-gamma agonists and antagonists were used in the cell models during the analyses.

Results. HECV cells weakly expressed PGC-1 and PPAR-gamma. Using the transgenes created, sublines over-expressing PGC1 (HECVPGC1exp) or with PGC-1 expression knockdown (HECVPGC1KD) were established. Loss of PGC1 showed marked increase in both adhesion and cellular migration, opposite was true for HECVPGC1exp cells. PPAR-gamma agonist, pioglitazone and ciglitizone, reduced the adhesion of control cells. In contrast, PPAR-gamma antagonist SR202 exhibited a concentration dependent stimulation of both adhesion and migration of the control cells. Knocking down PGC1 in endothelial cells rendered cells lost response to SR202.

Conclusions. PGC-1 is essential in the modulation of endothelial cells by PPAR-gamma. Together with the aberrant expression of both PPAR-gamma and PGC-1 in breast cancer, it is concluded that PGC-1 is a central player in PPAR-gamma mediated action in both cancer cells and angiogenesis.


P2-05-03

Is Breast Cancer Tryptase a Novel Anti-Angiogenic Molecular Target?

Tryptase, a serine protease stored and released from mast cells granules has been identified as a new non-classical angiogenic factor. Tryptase is an agonist of proteinase-activated receptor-2 a G protein involved in cellular proliferation and angiogenesis. On the other hand mast cells can release tryptase following c-Kit receptor activation. We have evaluated the correlations among the number of MCs positive to tryptase (MCDPT), the number of c-Kit receptor expressing cells (C-KREC) and microvascular density (MVD) in a series of 91 primary T1-3, N0-2 M0 female breast cancer by means of immunohistochemistry and image analysis methods. Six-micrometers thick serial sections of formalin-fixed and paraffin-embedded biotpic tumor samples were microwaved at 500 W for 10 min. and treated with a 3% hydrogen peroxide solution. Sections were incubated with primary antibodies: anti-trypotase (AA1; Dako, Glostrup, Denmark), anti-c-Kit receptor (A4502; Dako, Glostrup, Denmark) and anti-CD34 (QB-END 10; Bio-Optica Milan, Italy). In serial sections MVD, MCDPT and C-KREC were counted by means of image analysis (Quantimet 500 Leica) at x400 microscopic fields. Data demonstrated a significantly (r= ranging from 0.72 to 0.91; p: ranging from 0.001 to 0.003 by Pearson’s analysis respectively) correlation between MVD, MCDPT and C-KREC to each other. Published in vitro data suggest that tryptase induce angiogenesis in vascular endothelial cells and breast cancer cells lines. According to these data we shown that MVD, MCDPT and C-KREC paralleled to each other suggesting a role in vivo breast cancer angiogenesis. In this context the inhibition of c-Kit mast cells tryptase degradation by several tyrosin kinase inhibitors might be evaluated in clinical trials. Finally available tryptase inhibitors such as gabexate mesilate or nafamost mesilate might be evaluated in clinical trials as a new antiangiogenic approach.

P2-05-04

Mapping the Specific Gene Families Activated in the Lymphangiogenesis and Vasculogenic Mimicry Exhibited by Inflammatory Breast Cancer.
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Background: Inflammatory breast cancer (IBC) is the most metastatic variant of locally advanced breast cancer. Although IBC is diagnosed less commonly than other types of breast cancer, it is extremely aggressive, and accounts for a disproportionate number of breast cancer related deaths annually. IBC exhibits very specific patterns of lymphangiogenesis and vasculogenic mimicry, however detailed studies of the genes and proteins involved in these angiogenic processes are lacking. This study performed whole unbiased gene transcription studies with validation by protein arrays using all available pre-clinical cell lines and in vivo xenograft models of IBC; including a new model of IBC, FC-IBC01, which exhibits lymphovascular invasion, to identify the specific pathways involved in the distinctive angiogenesis observed in IBC.

Materials and Methods: Real-time quantitative RT-PCR, cDNA microarray gene profiling, immunofluorescence with confocal imaging and protein arrays were used to examine differential expression of specific angiogenic gene families including VEGF,A,B,C,D, VEGF Receptor genes, and ANG/TIE genes linked to angiogenesis and lymphangiogenesis.

Results: Activity of the matrix metalloproteinase, MMP-2, is required for IBC tumor cells to undergo vasculogenic mimicry (VM), which is associated with a loss of TIMP-2, a well known inhibitor of angiogenesis. Therapeutics that target MMP activity can successfully inhibit this VM. Furthermore, pre-clinical models of IBC that form IBC tumor emboli exhibit lymphovascular invasion that is associated
with distinct patterns of expression of genes that encode for distinct receptor tyrosine kinases that may represent important therapeutic targets for IBC.

Discussion: Identification of the distinct angiogenic pathways that are activated in IBC provides insight into the therapeutic targets that may abrogate the distinct lymphovascular invasion and vasculogenic mimicry that are linked to the aggressive metastasis of IBC.

**P2-05-05**
**Receptor-Like Protein Tyrosine Phosphatase Kappa (PTPRK) and Its Biological Role in Angiogenesis.**
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Introduction: Protein Tyrosine Phosphatases (PTPs) are known as signalling molecules which affect cell growth, differentiation and oncogenic transformation. PTPs have been indicated in tumourigenesis and progression of various solid tumours. PTPRK, receptor-type protein tyrosine phosphatase kappa, has been shown to down-regulate transcriptional activity of beta-catenin and impact distribution of beta-catenin/E-cadherin complexes on cancer cell membrane. However, the role played by PTPRK in angiogenesis remains unknown. In present study, the effect of PTPRK knock-down on functions and tubule formation ability of HECV cells was investigated. (Or in the present study, the effect of PTPRK on angiogenesis process was investigated.)

Methods: anti-PTPRK ribozyme transgenes were constructed to knock-down PTPRK expression in vascular endothelial cells, HECV cells. The subsequent effect upon in vitro cell growth, adhesion, migration and microvascular tubule formation was examined using a variety of functional assays.

Result: Knock-down PTPRK in HECV cells (HECVPTPRK) resulted in a decrease of cell growth in vitro. The growth rate of HECVPTPRK was 276.3±16.4, p=0.01 compared with HECVPTPRK (314.8±21.9) controls. However, knock-down of PTPRK in HECV cells increased cell motility. The cell migration distance of HECVPTPRK was 83.8±19.8µm, p=0.008 compared with HECVPTPRK (61.8±11.8µm) controls. No effect on cell adhesion by PTPRK knockdown was seen in HECVPTPRK (81.2±6.6), compared with HECVPTPRK control (78.8±15.6). Furthermore, knock-down of PTPRK suppressed tubule formation in HECV cells, the length of total tubules (µm) in HECVPTPRK was 2167.4±943.2, p=0.05 compared with HECVPTPRK (3130.6±386.9) control.

Conclusion: Knock-down of PTPRK reduced the growth and tubule formation abilities of vascular endothelial cells. It suggests that PTPRK is pivotal regulator for angiogenic process. Further investigations are required to identify the downstream pathways involved in these impacts.

**P2-05-06**
**Role of Repulsive Guidance Molecule b (RGMb) in HGF Mediated Angiogenesis.**
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Introduction: Hepatocyte Growth Factor (HGF) has been widely documented as playing a key role in enhancing the aggressive nature of cancer through its ability to promote cellular processes such as migration, invasion and angiogenesis. Development of a blood supply is vital to advanced tumour growth and increased metastatic potential. In the current study we identified RGMb (DRAGON), a member of the Repulsive Guidance Molecule family, as being upregulated by HGF and assessed its potential to contribute to HGF mediated pro-angiogenic traits.

Methods: Expression of RGMb was identified as being upregulated in human endothelial HECV cells following 4 hour treatment with HGF (40ng/ml) using micro array analysis and Q-PCR. Subsequently, the expression of RGMb was targeted through the transfection of HECV cells with a plasmid containing a ribozyme transgene specifically targeted to RGMb. The role of RGMb in HGF mediated cellular migration and tubule formation in vitro was examined.

Results: Significant increases in RGMb expression in HECV cells were observed on the micro-array following 4 hour treatment with HGF (P = 0.004) and this trend was also identified using Q-PCR. Transfection with the ribozyme transgene brought about substantial reductions in RGMb expression at both transcript and protein levels as assessed using RT-PCR, QPCR and Western blot analysis. Knockout of RGMb brought about a significant increase in migration rates, compared to HECV cells transfected with a closed pEF6 plasmid only (HECVpEF6), following a 90minute period (p = 0.034 at 90 minute time point vs pEF6 control). Treatment of HGF enhanced migration rates of HECV control cells with significant differences between untreated and HGF treated pEF6 cells obvious following 60 minutes (p < 0.05 at 60 and 75 minutes, p = 0.002 at 90 minutes). In contrast to this, HECV cells transfected with the RGMb ribozyme transgene (HECVRGMbKO) were unaffected by HGF treatment, with no significant differences observed between treated or untreated HECVRGMbKO cells at any time points. A similar trend was observed in the angiogenic tubule formation assay, where treatment of HECVRGMbKO cells with HGF could significantly enhance the levels of tubules formed (mean tubule perimeter, untreated 8087+/− 632 vs HGF treated 13131+/− 988, p = 0.001). Treatment of HECVRGMbKO cells with HGF did not significantly enhance levels of tubule formation (mean tubule perimeter, untreated 7523+/−2458 vs HGF treated 11050+/−1512, p = 0.21).

Conclusions: Targeting of RGMb in endothelial cells appears to reduce their sensitivity to the promotional effect of HGF on cell migration and tubule formation, important traits in the angiogenic cascade. Our data suggests that RGMb may be one molecule involved in the process through which HGF enhances angiogenic potential and targeting this molecule may be a useful strategy in a number of cancer types to interfere with HGF promoted angiogenesis.

**P2-05-07**
**Combined Antiangiogenic and Anti-Estrogen Therapy in Breast Cancer. Molecular Mechanisms Involved.**
de la Haba J, Berciano M, Cañabate M, Porras I, Valverde A, Cañas A, Rodríguez A, Aranda E. Hospital Universitario Reina Sofia, Instituto for Biomedical Research Maimonides IMIBIC, Cordoba, CO, Spain; Hospital General Universitario, Ciudad Real, CR, Spain

Background: Much is known about the mechanisms involved in the response to anti-hormonal treatment or those involved in the response to antiangiogenic therapy. It is also known the association between angiogenesis and hormonal status, both in physiological and pathological settings. However, the molecular and cellular mechanisms contributing to the efficacy of combined antiangiogenic-antihormonal therapy in breast cancer are still unknown. This
Combination is currently in clinical trials, but unfortunately there are scarce preclinical studies contributing to the rationale of combining antiangiogenic and antihormonal therapies.

**Aims:** To define the mechanisms involved in the response to combined antiangiogenic-antihormonal treatments in breast cancer cells.

**Methods:** Breast cancer cell lines with different estrogen dependence (MCF-7, BT-474, MDA-MB-231) were subjected to an estrogen gradient (estradiol), and treated with fulvestrant (antiestrogen), plus bevacizumab (anti-angiogenic). Cellular proliferation and apoptosis were determined using the corresponding kits. Proliferation and survival intracellular signaling pathways, estrogen alpha and VEGF receptors activation and COX-2 expression were analysed by western-blots using specific antibodies.

**Results:** In estrogen-dependent breast cancer cells (MCF-7 and BT-474) the pro-proliferative effect of estradiol decreased after bevacizumab treatment.

**Table 1. Proliferation 48 hours (%) ± SD**

<table>
<thead>
<tr>
<th>Breast Cancer Cell Lines</th>
<th>E</th>
<th>E+H</th>
<th>E+F</th>
<th>E+H+F</th>
<th>E+H+B</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCF-7 (ER+)</td>
<td>100±2.9</td>
<td>96±1.0</td>
<td>97.6±1.7</td>
<td>20.1±0.2</td>
<td></td>
</tr>
<tr>
<td>BT-474 (ER+)</td>
<td>100±3±16.3</td>
<td>96.5±0.6</td>
<td>95.9±6.2</td>
<td>29.2±0.2</td>
<td></td>
</tr>
<tr>
<td>MDA-MB-231 (ER-)</td>
<td>100±0.7</td>
<td>70.2±2.4</td>
<td>95±1±2.8</td>
<td>68.5±3.6</td>
<td></td>
</tr>
</tbody>
</table>

Furthermore, the combination of the antiangiogenic with an antiestrogen enhanced this antiproliferative effect, that was also related to the reduction in the levels of VEGF-A in the culture medium and to diminished ER alpha phosphorylation. The combined treatment also altered the phosphorylation of Akt and Erk1/2 signaling kinases. Interestingly, bevacizumab treatment augmented COX-2 activity in MCF-7.

**Conclusions:** Our results suggest that in estrogen dependent breast cancer cells the anti-proliferative effect of bevacizumab depends on estradiol concentration, that in turn affects VEGF production levels, using a different mechanism to apoptosis. The combination of bevacizumab with antioestrogens enhances this antimutator effect, altering intracellular signaling pathways of proliferation and survival.

**P2-05-08**

**Expression of VE-Statin/egfl7 in Breast Cancer.**

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**Patients and methods:** Specimens of breast cancer (174 invasive and 37 in situ) were obtained from 205 patients (pts) treated in our institution in 2005. Their median age was 57 years (39-92). VE-statin expression was studied by immunohistochemistry (IHC) using anti-hVE-statin/egfl7 (AF3638, R&D) and by RT-qPCR on formalin-fixed paraffin sections. 174 invasive carcinomas (141 ductular and 30 lobular) and 37 DCIS were studied. Determination was done twice by two different observers. Statistics used Chi2 and Fisher’s exact tests for categorical data and Kruskall-Wallis test for continuous variables.

**Results:** Pathological characteristics of the patients with invasive tumors were as follows: 81% ductular, 17% lobular; median tumor size 18 mm (2-100); SBR grade 1 in 27%, 2 in 58% and 3 in 15% of the tumors; estradiol receptor (ER+) in 90%, progesterone receptor (PR) + in 66%, 6.5% HER2 positive. In 105 cases there was DCIS together with invasive tumors. For invasive tumors and DCIS, progression-free survival (PFS) at 5 years were 83% and 100% and overall survival (OS) 96% and 95%, respectively. Characteristics of the DCIS were median tumor size 18 mm (2-60), ER+ in 88%, PR+ in 62%. IHC results were obtained in 159 invasive carcinoma and 34 DCIS, RT-qPCR results in 96 samples. In the group of invasive tumors, a cytoplasmic VE-statin/egfl7 signal was obtained in 80% of invasive cells, more frequently than in normal glandular cells (71% vs 41%, p=0.003) but significantly less than in peritumoral DCIS (71% vs 89%, p=0.001). The signal was higher in ductular than in lobular carcinoma (85% vs 54%, p=0.003). In RT-qPCR analyses, RNA levels were higher in SBR1 grade tumors (p=0.008), in ER+ invasive tumors (p=0.02) and in pN- tumors (p=0.003). In the group of DCIS, a cytoplasmic signal was obtained in 82% of the cells. It was more frequently observed in tumor than in normal glandular cells (82% vs 52%, p=0.02). A+ cytoplasmic signal was more frequent in DCIS alone than in DCIS associated with invasive carcinoma (23% vs 6%, p=0.02). No prognostic significance differences were observed on PFS or on OS.

**Discussion:** This work is the first expression study of VE-statin/egfl7 in breast cancer. The results were reproduced by two different observers. IHC signal was cytoplasmic and higher in DCIS than in invasive carcinomas. Furthermore the signal was even higher when there was only DCIS than when it was associated with invasive lesion. The signal was higher in ductular than lobular carcinoma, consistent with previous publications on other angiogenesis mediators. Similarly, the signal was higher in low SBR grade, as well in ER positive tumors. Phase 1 studies are ongoing in order to evaluate the effects of inhibition of VE-statin/egfl7.

**P2-05-09**

**Secondary Inflammatory Breast Cancer: A Possible Model for Post-Surgical Dissemination of Cancer.**

Hashmi S, Zolfaghari L, Levine PH. George Washington University, Washington, DC

**Background:** The phenomenon of accelerated tumor growth following surgery has been observed repeatedly and merits further study. Inflammatory breast carcinoma (IBC) is widely recognized as an extremely aggressive malignancy characterized by micrometastasis at the time of diagnosis. It is fast growing, highly angiogenic and angiogenic, features that are present from its inception. The idea of a dormant cancer cell and awakening of metastatic disease following a surgical/traumatic event may well be exemplified by secondary IBC, a term used to describe the IBC appearing following surgery for a non-inflammatory primary breast carcinoma. One possible mechanism can be related to the stimulation of dormant micrometastasis through local angiogenesis occurring as part of postsurgical healing. It is therefore possible that secondary IBC can be used as a model to support local angiogenesis as an important contributor to the development of an aggressive cancer.

**Materials and Methods:** Cases of secondary IBC were identified in a review of patients referred to the IBC Registry (IBCR). In this report we document the histories of three patients with secondary IBC as well as two additional patients whose disease presentation also supports the possible occurrence of IBC secondary to breast trauma. Secondary IBC cases were defined as women who had surgery for non-inflammatory breast cancer with recurrence at the previous mastectomy site manifest as skin erythema shown to be associated with angiogenesis occurring as part of posttraumatic healing. It is therefore possible that secondary IBC can be used as a model to support local angiogenesis as an important contributor to the development of an aggressive cancer.

**Results:** Two of the patients with secondary IBC developed pathologically confirmed dermal lymphatic invasion two and 42 months after partial mastectomy for non-inflammatory breast cancer. The third had been apparently free of recurrence for seven years when she had reconstructive surgery, which was followed by IBC seven months later. Two additional cases are presented, one in which IBC manifested one month following ductogram procedure and had a...
contralateral breast IBC recurrence 2 years later. The other patient was diagnosed with IBC one year following nipple piercing and ring removal.

Discussion: Recent publications have focused on the role of surgery in the subsequent development of metastatic breast cancer, many of them focusing on a hormonal mechanism triggered by removal of the primary tumor. We propose local angiogenesis as another possible mechanism for post-surgical dissemination of cancer. In view of the hypothesis that trauma can stimulate angiogenesis which can accelerate tumor growth, the documentation of IBC appearing at the site of a traumatic event merits consideration. Our experience with IBC, noted in the case reports above suggest that local trauma probably mediated in large part by angiogenesis can be an important trigger of IBC. We would therefore suggest that secondary IBC be considered for investigation of one possible mechanism for post-surgical tumor dissemination.

P2-05-10
Brain-Derived Neurotrophic Factor, BDNF, and Its Biological Impact on Vascular Endothelial Cells.
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Background: BDNF, Brain-derived neurotrophic factor is a member of the neurotrophin superfamily and is known a growth factor for both central and peripheral nervous systems. While BDNF has been shown to play a role in the development of a number of clinical conditions including epilepsy, Alzheimer’s and Parkinson’s disease, it has recently been implicated in human cancers, primarily in neurological related malignancies, as well as ovarian cancer, lung cancer and skin malignancies. We have recently reported a pivotal relationship between BDNF and disease progression and clinical outcome in human breast cancer. Certain members of the neurotrophin family have also been shown to play a role in the regulation of angiogenesis. In the present study, we examined the impact of BDNF on the biological behaviour of vascular endothelia cells.

Materials and methods. Human vascular endothelial cells were used in the present study. A human BDNF expression plasmid was constructed from a cDNA library of normal tissues. Anti-BDNF transgenes were constructed based on the secondary structure of human BDNF and used to knockdown the expression of BDNF from the cells. Vascular endothelial cells with differential expression of BDNF were generated using the expression construct. Cell-matrix adhesion, cell migration and growth were determined.

Results. Human vascular endothelial cells (HECV) expressed very low levels of BDNF, traceable by PCR method. The cells were transfected with BDNF expression plasmid, which allowed establishment of BDNF over-expressing sublines, HECVBDNFexp. Expression of BDNF had a marked influence on matrix adhesion of the endothelial cells (adhesion indices for control and HECVBDNFexp cells being 3.3± 0.83 and 2.18± 0.94, respectively; p=0.01). It is interesting to note that the change of adhesion was likely to involve the focal adhesion (FAK) pathway as a FAK inhibitor, PF573228, had a profound impact on the adhesion of both control and HECVBDNFexp cells. Manipulation of BDNF in endothelial cells had a similar effect on the cellular migration, although to a lesser degree. Conclusions. Brain-derived neurotrophic factor, BDNF, has a profound impact on the biological functions of vascular endothelial cells. This may have implications in the angiogenic process seen in solid tumours.

P2-06-01
Oikawa M, Yoshiura K-I, Kondo H, Miura S, Kurashige T, Nagayasu T, Yano H, Nakashima M. Atomic Bomb Disease Institute, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan; Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

Background: Sixty-six years after two atomic bombs (A-bombs) explosion on Hiroshima and Nagasaki, the incidence of breast cancer in A-bomb survivors still higher than that in controlled populations. We have reported a higher incidence of HER2 and C-MYC oncogene amplification in breast cancers from A-bomb survivors, which suggested genomic instability (GIN) induced by A-bomb radiation exposure might promote breast carcinogenesis in A-bomb survivors. The purpose of this study was to clarify the effect of A-bomb radiation exposure on GIN in breast cancer using microarray-comparative genomic hybridization (aCGH). Materials and Methods: DNA was extracted from the paraffin-embedded tissues of invasive ductal cancers from 11 survivors exposed at 1.5km from the hypocenter and 9 calendar year-matched non-exposed patients followed by aCGH analysis using a high-density oligonucleotide microarray (Agilent® SurePrint G3 8x60k microarray). The total length of chromosomal aberrant region was used as an indicator of GIN, and correlation with clinicopathological factors were statistically tested. Results: The mean of the derivative log ratio spread (DLRSpread), which estimates the log ratio noise by calculating the spread of log ratio differences between consecutive probes along all chromosomes, was 0.60 (range, 0.30 to 1.05). The concordance of aCGH results with FISH results for HER2 gene amplification was 90%. Samples from A-bomb survivors had significantly more copy number aberrations (CNA) than samples from control patients (P= 0.040). Samples with C-MYC gene amplification determined by aCGH analysis tended to harbor more CNA (P= 0.20), and age at the time of diagnosis tended to be inversely associated with the total length of chromosomal aberrant regions (P= 0.13). The multivariate analysis with covariance revealed that the status of A-bomb exposure was the only independent factor which was significantly associated the total length of chromosomal aberrant regions (P= 0.0324). Conclusions: This study suggested that A-bomb radiation may affect the development of chromosomal aberrant regions including oncogene amplification by inducing GIN and may be associated with a higher histological grade in breast cancer found in A-bomb survivors.

P2-06-02
FOXO3a Genotype Predicts Age of Breast Cancer Onset and Correlates with Lymph Node Involvement.

Background: Ageing mechanisms and genetic traits underlying the aging process are intimately linked with mechanisms that protect against cancer. FOXO3a genotype might be an important clue in this regard, as single nucleotide polymorphisms in this gene have been related to longevity, but also to differences in cancer susceptibility. FOXO3a is thought to work as a cellular ‘switch’, by either promoting apoptosis or halting proliferation and protecting from oxidative stress.

Survivors: An Analysis of Microarray-Comparative Genomic Hybridization with Old Archival Tissues.
We have assessed FOXO3a genotype in a cohort of breast cancer patients to investigate possible associations with patient and/or breast tumor characteristics. 

**Patients and methods:** Eligible subjects were all early breast cancer patients with upfront surgery, diagnosed at our center between 2000 till 2010, from which germline DNA (peripheral blood) was available. 2 SNP's in the FOXO3a gene, known to correlate with ageing or age associated diseases were assessed by Sequenom (rs 9400239 and rs 6911407).

**Results:** 2047 patients were included in the study. Age at diagnosis was found to be lower for patients homozygous for the minor allele at position rs6911407 (genotype A/A) (median age at diagnosis 52 years; P=0.001; 95% C.I. 50.7–53.3). Patients homozygous for the wild type allele (genotype C/C) at this position had a higher median age at diagnosis 55.0 years; 95% CI. 54.0–56.0. After correction for possible confounding variables BMI at diagnosis and parity, the association between age at diagnosis still remained significant (P=0.025, N=1348). We also assessed the relationship of the specific genotypes with tumor characteristics. We found that rs6911407 is positively correlated with lymph node status. This finding remains significant after correction for possible confounding variables ER, PR, Her-2, tumor size and tumor grade (OR=1.224 for homozygous mutants versus wild type; P=0.014; 95% C.I. 1.042–1.437).

**Conclusion:** In our cohort of primary breast cancers, we found that patients homozygous for the minor allele at position rs6911407 in the FOXO3a gene, develop breast cancer at younger age, and have more lymph node involvement compared to patients homozygous for the wild type allele. SNP's in nearly complete linkage disequilibrium with rs6911407 have previously been correlated with healthy longevity. At first sight, this seems contradictory with our current findings. However, our results are in accordance with the trade-off that has been described to exist between longevity and risk of cancer. It is generally assumed that FOXO3a and p53 function are oppositely controlled by common regulation systems, such that changes in FOXO activation would always be counterbalanced by an inverse change in p53 activation. If FOXO3a is strongly activated in order to protect against DNA damage (i.e. longevity function), p53-mediated cancer protection will concomitantly be diminished. This mechanism reconciles the apparent paradox between the longevity phenotype that has previously been linked with rs 6911407 on the one hand, and, on the other hand, our current findings of earlier breast cancer onset and increased lymph node metastasis. Further research on the function of FOXO3a in both aging and cancer is required to shed more light on the molecular mechanisms underlying these observations.

**P2-06-04**

Phosphatidylinositol-3-Kinase Pathway Mutations Are Common in Breast Columnar Cell Lesions.

Troxell ML, Brunner AL, Montgomery K, Zha SX, Neff T, Warrick A, Beadling C, Corless CL, West RB. Oregon Health & Science University, Portland, OR; Stanford University, Stanford, CA

The phosphatidylinositol-3-kinase pathway is one of the most commonly mutated in invasive breast carcinoma, with PIK3CA mutations present in ~25% of invasive carcinomas, and AKT1 mutations identified in up to 5%. Several studies have demonstrated the same complement of mutations in ductal carcinoma in-situ (DCIS), as well as benign papillomas. We sought to investigate whether PIK3CA mutations occur in breast columnar cell lesions (CCL). Twenty-five breast resection specimens containing CCL (including columnar cell change, columnar cell hyperplasia, and flat epithelial atypia) were identified from the files of Stanford University Pathology; 15 of these had associated invasive carcinoma (IDC) or carcinoma in situ. DNA was prepared from punchs of formalin-fixed paraffin-embedded tissue blocks using standard methods. DNA extracts were screened for a panel of point mutations using a multiplex PCR panel with a mass-spectroscopy readout (Sequenom MassARRAY). The panel covers 321 mutations in 30 genes, including ABL, AKT1/2/3, BRAF, CDK4, CTNNB1, EGFR1, ERBB2, FBX4, FBXW7, FGFR1/2/3, FLT3, GNAQ, HRAS, JAK2, KIT, KRAS, MAP2K1/2, MET, NRAS, PDGFR, PIK3CA, PTPN11, RET, SOS1, and TP53.

The majority of mutations were confirmed by Sanger sequencing. PIK3CA mutations were identified in 12/25 CCL (48%); paired normal breast tissue was tested in 21 cases and was negative for mutations in all but one case. In associated DCIS, 4/8 (50%) harbored PIK3CA mutations, while 3/9 IDC had mutations (33%; 2 PIK3CA, 1 AKT1). The mutation status of CCL and carcinomas was frequently discordant. Of 15 cases, only 6 demonstrated the same genotype in matched samples of CCL and carcinoma (5 wildtype, 1 PIK3CA H1047R). Interestingly, 5 patients had mutations in CCL with wildtype DCIS or IDC; 2 patients had different point mutations in CCL and carcinoma, including one patient with discordant mutant DCIS and wildtype IDC. Only 3 cases had wildtype CCL and mutated carcinoma.

**P2-06-03**


Grizzle WE, Steg AD, He Q, Stecuk MR, Byan-Parker S, Johnson MR, Grunda JM. University of Alabama at Birmingham, Birmingham, AL; Tuskegee University, Tuskegee, AL

Background: Recent studies suggest that the poorer outcome of breast cancer patients observed in African-American women (AAW) may, in part, result from underlying genetic factors. The purpose of this study was to investigate gene expression differences between Caucasian-American women (CAW) and AAW that may contribute to this poorer prognosis.

Methods: The expression of genes involved in breast carcinoma prognosis, response to therapy, estrogen signaling, and tumor aggressiveness was assessed in age- and stage-matched CAW and AAW paraffin-embedded breast cancer specimens. The Wilcoxon-Mann-Whitney Test was used to identify genes with a significant difference in expression between CAW and AAW. To determine if the differentially expressed genes could segregate between the CAW and AAW, we performed semi-supervised principle component analysis (SSPCA).

Results: Twenty genes were differentially expressed between AAW and CAW. SSPCA incorporating these 20 genes separated AAW and CAW into two distinct groups. AAW were significantly (p < 0.05) more likely to display aberrations in G1/S cell-cycle regulatory genes, decreased expression of cell-adhesion genes, and low to no expression of ESR1, PGR, ERBB2 and estrogen pathway targets.

Conclusions: The gene expression differences identified between AAW and CAW may contribute to more aggressive disease, resistance to therapy, enhanced metastatic potential and poor clinical outcome. Impact: These findings support the hypothesis that breast cancer specimens collected from AAW display distinct genetic differences compared to similar tissues obtained from CAW. Additional population-based studies are necessary to determine if these genetic variations contribute to the highly aggressive and treatment-resistant breast cancer phenotype frequently observed in AAW.
The nearly 50% PIK3CA mutation prevalence in CCL is greater than reported in most studies of invasive breast cancer. Further, CCL and carcinoma were frequently discordant for PIK3CA/PTEN mutation status; most commonly the CCL harbored a PIK3CA mutation, while the associated carcinoma was wildtype. Although these findings need validation in a larger study, they raise interesting questions as to the role of PIK3CA/PTEN pathway in breast carcinogenesis, and as to the biologic/precursor potential of CCL.

P2-07-01
Copy Number Variants in Early-Onset-Breast Cancer.
Ludwig S, Ivanovich J, Graubert TA, Goodfellow PJ. Washington University in St.Louis, SOM, St. Louis, MO

Background: Copy number variants (CNVs) are known to contribute to the risk for development of immune diseases, neurological disorders and to a lesser extent cancer. Many CNVs do not directly involve gene sequences. CHL1, a cell adhesion molecule with homology to L1CAM, is down-regulated in a large proportion of breast cancer. A deletion CNV immediately 5' of the CHL1 transcriptional start site has been described. The CHL1 CNV's role in breast cancer risk has not previously been explored.

Material and Methods: 50 research subjects recruited to the Washington University/Siteman Cancer Center Young Women's Breast Cancer Program (YWBCP) were selected for a CNV discovery study. These women were diagnosed breast cancer < age 40, tested negative for BRCA1/2 mutations and have a negative family history for breast cancer. The selection criteria enriched for de novo mutations. Comparative genomic hybridization (CGH) analyses were undertaken using the NimbleGen 2.1 million oligonucleotide array. DNA copy number was compared between proband and each of her parents to identify potential deletions or amplifications in the daughter relative to both parents (de novo changes). PCR was used to confirm a subset of the CGH-predicted differences in copy number. Chi-square test was used to determine statistical significances of CNV occurrences near the CHL1 gene between women with breast cancer and control cases.

Results: aCGH data analysis has been completed for 25 YWBC trios (patients and parents). 247 differences in copy number in the breast cancer cases relative to their parents were identified (1-18 putative de novo CNVs per case). 91% of the observed changes were deletions and 9% were amplifications. One proband showed an 11,560 bp deletion 5' of the CHL1 gene that was not seen in her parents. Conventional PCR confirmed a homozygous deletion of a ~3 kb region in the patient, in the region of a known CNV with a reported frequency of ~3%. Testing an additional 368 early-onset breast cancer cases for the CHL1 deletion revealed 25 additional homozygous deletions (6.8%). The deletion was present in 9 of 197 controls (4.5%). qPCR studies revealed the parents of the patient with the CHL1 deletion each carried a single copy of the CNV, making de novo copy number change in the proband very unlikely. Deletion specific assays for the CHL1 CNV are being used to test CNV genotypes in an extended case-control study.

Discussion: CHL1 expression has previously been shown to be down-regulated in primary breast tumors. Our preliminary analysis of YWBC cases and controls indicates the CHL1 regulatory region CNV is unlikely to be associated with risk for early-onset breast cancer. Additional candidate de novo copy number changes seen in the genomes of patients with early-onset breast cancer are being evaluated to determine their roles as genetic risk factors.

P2-07-02
Germline Genetic Variants Disturbing the Let-7/LIN28 Double-Negative Feedback Loop Alter Breast Cancer Susceptibility.
Yu K-D, Shao Z-M, Chen A-X. Fudan University Shanghai Cancer Center, Shanghai, China

Previous studies have shown that let-7 can repress the post-transcriptional translation of LIN28, and LIN28, in turn, could block the maturation of let-7, forming a double-negative feedback loop. In this study, we investigated the effect of germline genetic variants on regulation of the homeostasis of the let-7/LIN28 loop and breast cancer risk. We initially demonstrated that the T/C variants of rs3811463, a single nucleotide polymorphism (SNP) located near the let-7 binding site in LIN28, could lead to differential regulation of LIN28 by let-7. Specifically, the C allele of rs3811463 weakened let-7-induced repression of LIN28 mRNA, resulting in increased production of LIN28 protein, which could in turn downregulate the level of mature let-7. This effect was then validated at the tissue level in that the normal breast tissue of individuals with the rs3811463-TC genotype expressed significantly lower levels of let-7 and higher levels of LIN28 protein than those individuals with the rs3811463-TT genotype. Because previous in vitro and ex vivo experiments have consistently suggested that LIN28 could promote cellular transformation, we then systematically evaluated the relationship between rs3811463 as well as other common LIN28 SNPs and the risk of breast cancer in a stepwise manner. The first hospital-based association study (n = 2,300) demonstrated that two SNPs were significantly associated with breast cancer risk, one of which was rs3811463, while the other was rs6697410. The C allele of the rs3811463 SNP corresponded to an increased risk of breast cancer with an odds ratio (OR) of 1.25 (P = 0.0091), which was successfully replicated in a second independent study (n = 1,156) with community-based controls. The combined P-value of the two studies was 8.0 × 10-5. Taken together, our study demonstrates that host genetic variants could disturb the regulation of the let-7/LIN28 double-negative feedback loop and alter breast cancer risk.

P2-07-03
Collective Evidence Suggests Neutrality for BRCA1 V1687I, a Novel Sequence Variant in the Conserved THV Motif of the First BRCT Repeat.
Cortesi L, De Nicoló A, Medici V, Marino M, Turchetti D, Pradella LM, Rossi G, Parisini E, Federico M. University of Modena and Reggio Emilia, Modena, Italy; Dana-Farber Cancer Institute, Boston, MA; Harvard Medical School, Boston, MA; University of Bologna, Bologna, Italy; Italian Institute of Technology, Milan, Italy; These Authors Contributed Equally to This Work

Background: Unequivocal classification of BRCA1 and BRCA2 variants of uncertain significance (VUS) with respect to their effect on protein function is a challenge with consequent implications for clinical decision-making. Currently, integration of different types of evidence derived from independent sources appears to be the most reliable approach to help define disease relevance for each single VUS. During a routine screening for BRCA1 and BRCA2 mutations carried out by the Modena study group for familial breast and ovarian cancer we identified a novel BRCA1 sequence change (c.5178G>A) that causes a valine to isoleucine (V to I) substitution at position 1687. The sequence alteration was found in a patient diagnosed with early onset breast cancer and it was present neither in other breast/ovarian cancer families screened nor in unaffected control individuals. No co-occurrence of the variant with any known clinically relevant BRCA1 mutations was observed.
The C-terminally located BRCA1 V1687I lies in the conserved THV motif of the first BRCT repeat. Disease-causality has previously been suggested for two neighbouring sequence alterations, BRCA1 V1688del, whose repercussions on protein stability and function were also reported (De Nicolo et al., 2009), and BRCA1 H1686Q (Giannini et al., 2008). We aimed at garnering evidence that, together with family and genetic data, could help assess the clinical significance of the V1687I variant.

Methods: We employed a multimodal approach that included immunohistochemistry on tumor sections, in silico analyses, comparative structural modeling, and ascertainment of BRCT-mediated functional associations.

Results: Whereas tumor histology, receptor and HER2/neu status were compatible with a BRCA1-deficient phenotype, most algorithms we used to assess sequence and structure variation based on evolutionary conservation predicted the BRCA1 V1687I variant to be benign. In line with this prediction, analysis of the modeled BRCA1 V1687I BRCT domain did not reveal any major structural changes relative to the wild type counterpart and suggested that BRCA1 V1687I leaves the local architecture and overall stability of the protein largely unaltered. Consistently, the BRCA1 V1687I protein was properly expressed and localized to the nucleus, and it was still capable of binding BRIP1/FANCJ, ChiP and Abraxas, three BRCT-interacting, DNA damage response and repair partner proteins.

Conclusions: Based on our collected evidence, the BRCA1 V1687I amino acid change, although occurring in an evolutionary conserved region, does not seem to affect the function of the correspondingly encoded protein, and hence it is a likely benign sequence alteration.

Impact of Aromatase Inhibitors on Background Parenchymal Enhancement and Amount of Fibroglandular Tissue on Breast MRI.

Goldfarb SB, King V, Sung J, Pike M, Nulsen B, Jozefara J, Hudis C, Morris E, Dickler M. Memorial Sloan-Kettering Cancer Center, New York, NY

Background: On breast MRI, background parenchymal enhancement (BPE) and volume of fibroglandular tissue (FGT) have been shown to reflect a patient's hormonal status. Tamoxifen has been shown to reduce mammographic breast density and may serve as an early predictor of response in the prevention setting (Cuzick, JNCI 2011). We have shown that adjuvant tamoxifen can reduce BPE in the unaffected breast with breast cancer. We hypothesize that aromatase inhibitor (AI) induced endocrine changes in breast tissue should also be evident and therefore we performed a study to evaluate whether adjuvant AI therapy influences BPE or amount of FGT in the contralateral breast.

Methods: An electronic medical record review identified 856 postmenopausal women with stage I-III breast cancer who had at least two breast MRIs and took adjuvant AI treatment. A retrospective chart review was conducted to select those patients without a history of prior tamoxifen or raloxifene treatment who had a MRI of the contralateral breast both before and during 6 to 12 months of AI treatment. After exclusion of all irradiated breasts, 168 women were eligible. MRIs were performed between August 1999 and June 2010. Two radiologists who were blind to AI treatment status, independently rated level of BPE and amount of FGT using categorical scales: BPE – Minimal, Mild, Moderate, Marked; FGT – Fatty, Scattered, Heterogeneously Dense, Dense (based on proposed BI-RADS criteria for BPE and on ACR criteria for FGT). Blinded side-by-side direct comparison evaluated whether there was a category change between the two MRIs. A consensus was reached in cases of disagreement. The Wilcoxon signed-rank test was used to assess changes in rating categories for BPE and FGT between before and during AI breast MRIs. A waiver of authorization was granted by the institutional review board for this study.

Results: In this study 127/168 (76%) women were treated with anastrozole, 33/168 (20%) with letrozole and 8/168 (5%) with exemestane. Based on the blinded side-by-side comparison, a category (or more) decrease in BPE occurred during treatment with AIs (p=0.0001). There was an overall shift from higher to lower degree of BPE in 35% (45/127) of the women taking anastrozole while a category increase occurred in only one woman (1%; p <0.0001). A similar result was seen in the women taking letrozole [45% (15/33) had a decrease versus 3% (1/33) an increase; p=0.0003] and exemestane [25% (2/8) had a decrease versus 12.5% (1/8) an increase; p=0.50]. For FGT a category decrease occurred in 5% (6/127) of anastrozole users while no increase occurred [0% (0/127); p=0.016]. The respective numbers for letrozole were 3% (1/33) and 0% (0/33), and nobody on exemestane had a change in FGT.

Conclusions: After 6 to 12 months of treatment with adjuvant AIs, there was a statistically significant category (or more) decrease in BPE. BPE is more sensitive than FGT to changes in normal breast stroma that occur during adjuvant treatment with AIs and BPE may be a marker of anti-hormonal activity in the breasts.

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Goldfarb SB, King V, Sung J, Pike M, Nulsen B, Jozefara J, Hudis C, Morris E, Dickler M. Memorial Sloan-Kettering Cancer Center, New York, NY

Background: On breast MRI, background parenchymal enhancement (BPE) and volume of fibroglandular tissue (FGT) have been shown to reflect a patient’s hormonal status. Tamoxifen has been shown to reduce mammographic breast density and may serve as an early predictor of response in the prevention setting (Cuzick, JNCI 2011). We have shown that adjuvant tamoxifen can reduce BPE in the unaffected breast with breast cancer. We hypothesize that aromatase inhibitor (AI) induced endocrine changes in breast tissue should also be evident and therefore we performed a study to evaluate whether adjuvant AI therapy influences BPE or amount of FGT in the contralateral breast.

Methods: An electronic medical record review identified 856 postmenopausal women with stage I-III breast cancer who had at least two breast MRIs and took adjuvant AI treatment. A retrospective chart review was conducted to select those patients without a history of prior tamoxifen or raloxifene treatment who had a MRI of the contralateral breast both before and during 6 to 12 months of AI treatment. After exclusion of all irradiated breasts, 168 women were eligible. MRIs were performed between August 1999 and June 2010. Two radiologists who were blind to AI treatment status, independently rated level of BPE and amount of FGT using categorical scales: BPE – Minimal, Mild, Moderate, Marked; FGT – Fatty, Scattered, Heterogeneously Dense, Dense (based on proposed BI-RADS criteria for BPE and on ACR criteria for FGT). Blinded side-by-side direct comparison evaluated whether there was a category change between the two MRIs. A consensus was reached in cases of disagreement. The Wilcoxon signed-rank test was used to assess changes in rating categories for BPE and FGT between before and during AI breast MRIs. A waiver of authorization was granted by the institutional review board for this study.

Results: In this study 127/168 (76%) women were treated with anastrozole, 33/168 (20%) with letrozole and 8/168 (5%) with exemestane. Based on the blinded side-by-side comparison, a category (or more) decrease in BPE occurred during treatment with AIs (p=0.0001). There was an overall shift from higher to lower degree of BPE in 35% (45/127) of the women taking anastrozole while a category increase occurred in only one woman (1%; p <0.0001). A similar result was seen in the women taking letrozole [45% (15/33) had a decrease versus 3% (1/33) an increase; p=0.0003] and exemestane [25% (2/8) had a decrease versus 12.5% (1/8) an increase; p=0.50]. For FGT a category decrease occurred in 5% (6/127) of anastrozole users while no increase occurred [0% (0/127); p=0.016]. The respective numbers for letrozole were 3% (1/33) and 0% (0/33), and nobody on exemestane had a change in FGT.

Conclusions: After 6 to 12 months of treatment with adjuvant AIs, there was a statistically significant category (or more) decrease in BPE. BPE is more sensitive than FGT to changes in normal breast stroma that occur during adjuvant treatment with AIs and BPE may be a marker of anti-hormonal activity in the breasts.

Magnetic Resonance Imaging as a Predictor of Pathologic Response in Patients Treated with Neoadjuvant Systemic Treatment for Operable Breast Cancer (TBCRC 017).

De Los Santos J, Cantor A, Mcguire K, Golshan M, Meric-Bernstam F, Horton J, Nanda R, Amos K, Forero A, Hudis C, Meszoely I, Hwang S. University of Alabama at Birmingham, Birmingham, AL; University of North Carolina Chapel Hill, Chapel Hill, NC; Dana-Farber Cancer Institute, Boston, MA; Duke University Medical Center, Durham, NC; Memorial Sloan Kettering Cancer Center, New York, NY; University of Pittsburgh Medical Center, Pittsburgh, PA; MD Anderson Cancer Center Houston, TX; Vanderbilt University, Nashville, TN; University of Chicago, Chicago, IL; University of California at San Francisco, San Francisco, CA

Background: Increased pathologic complete response (pCR) rates observed with neoadjuvant chemotherapy for invasive breast cancer has prompted interest in whether patients with pCR can be identified preoperatively and potentially spared the morbidity of surgery. This multicenter retrospective study was performed to determine the accuracy of preoperative MRI in predicting pCR in the breast and whether MRI performance differs by molecular subtype, histology, and treatment regimen.

Methods: 770 women from 8 institutions were retrospectively identified as having received neoadjuvant systemic therapy with MRI obtained at baseline and after completion of systemic treatment. Tumor phenotypes were defined on the basis of estrogen and progesterone receptor (ER/PR or HR) and HER2 receptor status. Univariate and multivariate analyses of factors influencing radiographic complete response (rCR) and pCR were recorded, with rCR defined as resolution of any abnormal enhancement, mass, or distortion on MRI, and pCR defined as resolution of both invasive disease and DCIS.
Results: rCR and pCR for the total group were 182/746 (24%) and 179/746 (24%), respectively, with the highest rate of pCR seen among the triple-negative (TN; 57/155; 37%) and HR-/HER2+ (38/101; 38%) subtypes. Covariates significantly associated with rCR included T stage (p = 0.0002), tumor grade (p = 0.005), IHC phenotype (p = 0.005), and chemotherapy regimen (p = 0.0001). On multivariate analysis, only tumor phenotype was independently associated with likelihood of rCR, with both TN (OR = 2.00, 95% CI 1.20–3.33) and HR-/HER2+ (OR = 2.30, 95% CI 1.09–4.83) more likely to achieve rCR than HR+HER- (reference group). Overall accuracy of MRI for prediction of pCR was 74%. Sensitivity, NPV, PPV, and accuracy differed significantly among tumor subtypes, with the greatest NPV in the HR-/HER2+ and TN subtypes (Table 1). Among patients with rCR, ER-, status (OR = 6.4, 95% CI 1.1 to 35.6), PR-status (OR = 3.8, 95% CI 1.2 to 11.4), and tumor grade of 3 vs 1 or 2 (OR = 2.49, 95% CI 1.22–5.07) were independently associated with likelihood of rCR.

Discussion: MRI performance for predicting pCR in patients with invasive breast cancer receiving neoadjuvant systemic therapy differed significantly among breast cancer subtypes; however, this difference is likely due to subtype differences in frequency of pCR and not to intrinsically better or worse MRI detection. The relatively low NPV of MRI following neoadjuvant systemic therapy does not support using MRI rCR alone to accurately identify those patients that can safely avoid surgery.

Performance of Post-treatment MRI in the Breast

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Rate pCR (%)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>NPV (%)</th>
<th>PPV (%)</th>
<th>Accuracy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Group</td>
<td>179/746 (24)</td>
<td>75/179 (47)</td>
<td>85/179 (47)</td>
<td>85/182 (47)</td>
<td>470/564 (83)</td>
<td>555/746 (74)</td>
</tr>
<tr>
<td>HR-HER2+</td>
<td>44/327 (13)</td>
<td>52/93 (56)</td>
<td>43/232 (46)</td>
<td>44/60 (73)</td>
<td>243/283 (86)</td>
<td>263/327 (80)</td>
</tr>
<tr>
<td>HR-HER2-</td>
<td>11/113 (9)</td>
<td>9/11 (82)</td>
<td>11/102 (10)</td>
<td>10/60 (60)</td>
<td>15/113 (13)</td>
<td>30/175 (17)</td>
</tr>
<tr>
<td>HER-2-</td>
<td>27/148 (18)</td>
<td>86/111 (77)</td>
<td>87/138 (64)</td>
<td>84/45 (49)</td>
<td>30/148 (21)</td>
<td>91/178 (52)</td>
</tr>
<tr>
<td>TN-</td>
<td>57/155 (37)</td>
<td>98/98 (81)</td>
<td>28/57 (49)</td>
<td>28/67 (40)</td>
<td>98/168 (73)</td>
<td>107/155 (69)</td>
</tr>
</tbody>
</table>

p value* < 0.0001 NS 0.02 0.001 0.01

*p values compare the 4 phenotypes for pCR rate and for each performance measure with breast MRI

P2-08-04

Computer-Derived Breast MRI Features Have Complementary Value for Preoperative Selection of Systemic Drug Therapy in Node-Negative Stage-I/II Breast Cancer Patients.

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Background: Typical guidelines for adjuvant systemic drug therapy require assessment of lymph node status, tumor extent and grade. Increasing interest exists, however, for preoperative therapy (neoadjuvant drug therapy and preoperative partial breast irradiation).

Here, representative excision specimens are not available to assess eligibility for systemic therapy. Moreover, tumor grade may be difficult to assess in core biopsies resulting in underestimation rates up to 40%. Contrast-enhanced MRI has been shown to correlate with underlying tumor biology, but it is unknown whether it can complement eligibility criteria for systemic drug therapy.

Purpose: To assess the complementary value of MRI to determine eligibility for systemic drug therapy prior to breast-conserving surgery in node-negative stage-I/II breast cancer patients.

Materials and methods: Patients with preoperative node-negative invasive breast cancer ≥1.0 cm and eligible for breast-conserving therapy after conventional breast imaging and MRI were consecutively included between February 2000 and March 2007. Informed consent was obtained from all patients. Eligibility to systemic therapy was determined on the basis of national guidelines using age, size, tumor grade, as well as axillary lymph node status at final pathology. Twenty temporal and morphological features of contrast uptake at MRI were automatically analyzed by a previously build CAD workstation.

Preoperative lymph node status was derived from ultrasound-guided fine-needle aspiration. Multivariate logistic regression and receiver-operating characteristics (ROC) analysis was used to assess associations between preoperative features (age, ER status, computer-
Purpose: To investigate the effect of bevacizumab infusion on vascular parameters available through dynamic contrast-enhanced magnetic resonance (DCE-MR) imaging and to test their association with pathological response in primary breast cancer.

Materials and Methods: 73 patients (median age, 47 yrs; age range, 29-70 yrs) with biopsy-proven, previously untreated, primary breast cancer were recruited from October 2009 to November 2010 in this phase II, multicenter and non-randomized clinical trial. Patients (pts) received single infusion of bevacizumab (15 mg/kg) (C1) 3 weeks prior to the beginning of neoadjuvant chemotherapy consisting of 4 cycles of docetaxel (60 mg/m²), doxorubicin (50 mg/m²) and bevacizumab (15 mg/kg) every 21 days (C2-C5) following by 4 cycles of docetaxel (60 mg/m²), doxorubicin (50 mg/m²) and paclitaxel (200 mg/m²). DCE-MR imaging was performed before (n=72) and after (n=71) C1. K(trans), K(ep), V(e) and IAUGC(60) values were significantly different at the baseline and after C1 (p<0.01). Median changes were, respectively, -51, -101, -52.5 and -4.8. Fifty-two (74%) pts achieved response (G3-G4-G5) after C5 whether 18 (24%) were considered as no responder (G1-G2); for 3 (4%) patients Miller/Payne tumor evaluation was not available. At univariate analysis, negative estrogen receptor (ER) status and higher post-C1 K(ep) (p=0.057) showed a trend toward an association with response. At multivariate analysis, only ER status remains a significant predictor of response (p=0.04).

Conclusion: Computer-analysis of contrast-enhanced MR images has potential to complement preoperative selection of stage I/II node-negative breast-cancer patients for systemic drug therapy.

Reference:
1. Deurloo et al., Radiology 2005; 234: 693-701
2. Gilhuijs et al., Radiology 2002; 225: 907-916
3. De Navarra; Roche Basel; Roche Madrid
4. Menlo Park, CA
5. GE Healthcare, Menlo Park, CA
6. University of California San Francisco, San Francisco, CA
7. Stanford University, Stanford, CA
8. University of California San Francisco, San Francisco, CA; GE Healthcare, Menlo Park, CA
9. Hospital de Navarra, Pamplona, Navarra, Spain; Hospital Marques de Valdecilla; Hospital Civil de Basauri; Onkologoia; Hospital General Yagues de Burgos; Hospital Arnau de Vilanova de Lleida; Hospital Miguel Servet; Hospital de San Millan, Logroño; Hospital Donostia; Hospital de Navarra; Roche Basel; Roche Madrid

P2-08-05 Use of Dynamic Contrast-Enhanced MR Imaging To Predict Pathological Response in Primary Breast Cancer.

Boni V, Pina LJ, Hernandez B, Lopez-Vega JM, Calvo EG, Plaizuela A, Morales S, Anton A, Sanchez-Gomez RM, Alvarez I, Ilarramendi JJ, De Juan A, Martinez P, Llongart A, La Huerta A, Dominguez I, Garcia-Vellioso MJ, Garcia-Gonzalez M, Lao Romera J, Puertolas T, Scherer S, Sabarz L, Garcia-Foncillas J, Clinic University of Navarra, Pamplona, Navarra, Spain; Hospital Marques de Valdecilla; Hospital Civil de Basauri; Onkologoia; Hospital General Yagues de Burgos; Hospital Arnau de Vilanova de Lleida; Hospital Miguel Servet; Hospital de San Millan, Logroño; Hospital Donostia; Hospital de Navarra; Roche Basel; Roche Madrid

Purpose: To investigate the effect of bevacizumab infusion on vascular parameters assessed by dynamic contrast-enhanced magnetic resonance (DCE-MR) imaging and to test their association with pathological response in primary breast cancer.

Materials and Methods: 73 patients (median age, 47 yrs; age range, 29-70 yrs) with biopsy-proven, previously untreated, primary breast cancer were recruited from October 2009 to November 2010 in this phase II, multicenter and non-randomized clinical trial. Patients (pts) received single infusion of bevacizumab (15 mg/kg) (C1) 3 weeks prior to the beginning of neoadjuvant chemotherapy consisting of 4 cycles of docetaxel (60 mg/m²), doxorubicin (50 mg/m²) and bevacizumab (15 mg/kg) every 21 days (C2-C5) following by surgery. All pts underwent DCE-MR imaging before and 14-21 days after C1. Quantitative and semiquantitative kinetic parameters were calculated at baseline and after C1, including the volume transfer constant (K(trans)), which primarily reflects the wash-in of the contrast agent, the bloodflow rate constant (K(ep)), extracellular volume fraction (V(e)) and the initial area under the gadolinium concentration-time curve over 60 seconds (IAUGC(60)). Changes in the DCE-MRI kinetic parameters K(trans), K(ep), V(e) and IAUGC(60) were calculated and Wilcoxon test was used to assess significant effects induced by bevacizumab on kinetic parameters.

Pathological response on surgical specimens after C5 was assessed according to Miller and Payne classification. Pts with tumor reduction >30% were considered as responders (G3-G4-G5) whether tumor reduction <30% were considered as no responders (G1-G2). DCE-MR imaging parameters and clinical-pathological characteristics were correlated with pathological response using Mann-Whitney test in univariate and logistic regression in multivariate analyses. Receiver operating curves (ROC) was used to define the best cut-off of the parameter found associated with pathological response.

Results: DCE-MRI was performed before (n=72) and after (n=71) C1. K(trans), K(ep), V(e) and IAUGC(60) values were significantly different at the baseline and after C1 (p<0.01). Median changes were, respectively, -51, -101, -52.5 and -4.8. Fifty-two (74%) pts achieved response (G3-G4-G5) after C5 whether 18 (24%) were considered as no responder (G1-G2); for 3 (4%) patients Miller/Payne tumor evaluation was not available. At univariate analysis, negative estrogen receptor (ER) status and higher post-C1 K(ep) (p=0.057) showed a trend toward an association with response. At multivariate analysis, only ER status remains a significant predictor of response (p=0.04).

Conclusion: Bevacizumab affects tumor vasculature, perfusion and permeability as shown by the significantly reduction in all kinetic vasculature parameters obtained in DCE-MR imaging after C1. However, in our population these changes were not associated with pathological response. On the other hand, backflow rate constant, K(ep), a perfusion-related parameter derived from DCE-MRI yielded after C1 major than 80, may be associated with higher pathological response with a specificity of 88% and sensitivity of 90%. Future studies are warranted to confirm these findings.

P2-08-06 Improved Spatial Resolution Diffusion-Weighted Imaging for Characterizing Tumors and Treatment Response in Patients with Invasive Breast Cancer.


Background: Diffusion weighted magnetic resonance imaging (DWI) is a non-invasive technique that is sensitive to tissue microstructure. Previous studies have shown that DWI adds positive predictive value in diagnostic studies of breast cancer and it has been shown to predict tumor response to neoadjuvant chemotherapy. While DWI shows promise for evaluating breast cancer, the technique suffers from limitations. Specifically, image distortion is common with the echo planar sequence available for DWI on clinical scanners, and spatial resolution is lower than that of other MRI sequences. The group has optimized a high-resolution reduced field-of-view DWI acquisition, originally developed for the spine by Saritas et al., for breast imaging. The goal of this work was to compare high resolution (hr)-DWI to standard resolution (std)-DWI for characterizing breast tumors.

Methods: Patients undergoing neoadjuvant chemotherapy were scanned with MRI before and after one cycle of chemotherapy. Apparent diffusion coefficient (ADC) maps were calculated from hr-DWI and std-DWI data using previously described methods. One tumor region of interest (ROI) was defined on the hr-DWI slice estimated to contain the largest tumor area. This tumor area ROI was then applied to the corresponding slice and location on the std-DWI and hr-DWI ADC maps. Mean tumor ADC as well as 15th,
25th, 50th, 75th, and 90th percentile ADCs were calculated for both DWI acquisitions for all subjects.

Results: The mean tumor ADC values measured prior to treatment were similar for the hr-DWI and std-DWI acquisitions, however there was a significant difference between hr- and std-DWI 15th and 25th percentile ADC values ($p=0.0495$, $p=0.0717$) For the early treatment time point, significant differences between the two DWI acquisitions were found: mean tumor ADC, 15th, 25th, and 50th percentiles ($p=0.0302$, 0.0075, 0.0212, and 0.0488, respectively), with the most significant difference found for the lowest (15th) percentile measured. Tumor hr-DWI ADCs were consistently lower than std-DWI ADCs.

Discussion: These data show that although the mean ADC values calculated from the pre-treatment hr-DWI and std-DWI are similar, the lower percentile (15th, and 25th) ADC values are significantly lower for the hr-DWI acquisition. Our results also showed larger difference in lower percentile ADC values after the two sequences after one cycle of chemotheraphy. The differences in the lower percentile ADC values calculated from the hr-DWI are consistent with reduced partial voluming between viable tumor tissue, which is characterized by high ADC values, and normal fibroglanular tissue. This may be particularly important for post-treatment ADC measurements where tumor size may decrease, potentially making partial volume effects more pronounced. Continuing studies are evaluating the relationship between low percentile ADC values from hr-DWI and tumor stage and response to treatment.

**P2-08-07**
Ex-Vivo High Resolution MRI of Sentinel Lymph Nodes Following Subcutaneous Injection of Superparamagnetic Iron Oxide Nanoparticles.

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Background: Superparamagnetic iron oxide (SPIO) enhanced axillary MRI is a promising novel imaging modality that could be used to characterize sentinel nodes non-invasively. In order to better characterize lymph nodes and evaluate their morphological features, we imaged nodes ex-vivo using high resolution MRI, following a pre-operative subcutaneous injection of SPIO.

Material and methods: Prior to surgery patients received a circumareolar injection of SPIO (Endorem, Guerbet, Paris) into the upper outer quadrant of the affected breast. Sentinel lymph nodes excised during surgery were transported to the imaging department fresh. Nodes were placed in glass tubes and scanned using a Bruker 9.4T MRI system (T2 mapping: turbo spin echo, 16 equi-spaced TEs from 7.1 to 113.6 ms, TR = 1616 ms. T2* mapping: gradient echo, 6 equi-spaced TEs from 3.5 to 21 ms, TR = 1000 ms, flip angle = 30 degrees. Diffusion-weighted: spin echo, b-values 0 and 1000 s/mm2, TE = 18 ms, TR = 6500 ms.). Slice thickness was 1 mm with an in plane spatial resolution of 100μm. Image analysis was undertaken using OsiriX (v3.8, 64-bit).

Results: A total of 40 nodes were successfully imaged, excised from 14 patients. The internal architecture of nodes was clearly seen and in the 3 involved nodes, a macrometastasis was identified. Three patients received a 4ml injection of SPIO and 11 patients, a 2ml injection. More SPIO deposition was seen within nodal sinuses following 4ml of SPIO, but there was no significant decrease in mean T2 value. Four nodes were re-imaged following formalin fixation and nodal architecture was unchanged with a trend towards an increase in mean T2 values within nodes. SPIO was identified in all sentinel nodes.

Discussion: Ex-vivo MRI with subcutaneous SPIO contrast, is a useful method for imaging and characterization of sentinel nodes. A better understanding of ex-vivo features is a useful aide to understanding the morphological features seen following in-vivo SPIO enhanced axillary MRI.

**P2-08-08**
Benefit of Preoperative Breast MRI in Patients with Newly Diagnosed Breast Cancer.

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Objective: The aim of our study was to evaluate the effect of routinely performed preoperative Breast MRI (pMRI) on the planned therapeutic management of all patients with newly diagnosed Breast cancer.

Subjects and Methods: 180 patients with newly diagnosed Breast cancer between 9/2009 – 12/2010 were enrolled in this retrospective study performed in a certified European Breast Cancer Unit; mean age 60,6 years; 1,5 T MRI (TIM-Symphony, Siemens Germany). Results: In 53/180 (29,4%) cases the preoperative MRI changed the planned therapeutic management concerning surgical and chemotherapeutical options. In 42/180 (23,3%) patients additional lesions were identified only by pMRI, 33/42 (78,6%) have been malignant and 8/42 (19%) were benign. The real extent of the tumor was detected in 43/53 (81%) cases – group A; a bilateral carcinoma was detected in 4/53 (7,5%) cases – group B; in 6/53 (11,5%) cases the malignant lesion could only be detected with preoperative MRI - group C; for group A the pMRI led to wider excisions with 15 segmental - or quadrant resections and 28 mastectomies; for group B the pMRI resulted in 4 additional lumentectomies; for group C the pMRI caused 4 lumentectomies, 1 segmental resection and 2 mastectomies. 5 of 6 patients got neoadjuvant chemotherapy only due to results of pMRI; in 3 of these 5 patients breast conserving surgery was possible. Histology: DCIS 23/53 (43,4%), invasive cancer 23/53 (43,4%), multicentric/multifocal malignant lesions 7/53 (13,2%). The sensitivity of the pMRI was not influenced by the histology of the lesion, the age of the patient or menopausal status. The maximum periode between initial presentation of the patient and breast surgery was 21 days.

Conclusion: In 29,4% of our patients the pMRI resulted in a significant change of the planned therapeutic regime. Histology of the lesion, patients age or menopausal status did not influence the sensitivity of pMRI. There was no relevant delay in the initiation of treatment. Therefore we recommend performing pMRI in breast cancer staging routinely.

**P2-08-09**
The Impact of Preoperative Breast MRI in Newly Diagnosed Breast Cancer. A Prospective Study of Treatment Outcome in Patients Selected for Breast-Conserving Surgery in a Norwegian Multidisciplinary Breast Cancer Clinic.

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Background: The aim of the study was to evaluate the impact of preoperative breast MRI on primary surgical treatment in a specialized multidisciplinary breast cancer clinic. The diagnostic tools routinely used are conventional mammography, ultrasound with core needle biopsy, and clinical examination including palpation of breast and...
axillary nodes. If indicated, the examination includes ultrasound examination and fine needle biopsy of axillary glands. We offer all patients selected for breast-conserving surgery preoperative breast MRI.

Material and methods: We included consecutively 145 breast cancer lesions in 143 women (two had bilateral cancer) aged 35-75 in a prospective study, collecting data from January 2009 to December 2010. The patients were all selected for breast-conserving surgery based on the criteria recommended by the Norwegian Breast Cancer Group. That is, tumor ≤ 4 cm, or acceptable tumor:breast ratio, and age > 35. Multifocal lesions, defined as more than one tumor > 1 cm apart, size of tumor > 4 cm, or a large tumor:breast ratio, extensive ductal carcinoma in situ (DCIS), or known genetic disposition for breast cancer, indicates mastectomy according to these criterias. In addition, when postoperative radiation therapy is contraindicated, mastectomy is indicated. If the patients filled the criteria for breast-conserving surgery, they were offered preoperative breast MRI and included in the study. Patients who either choose mastectomy, or did not get a breast MRI for technical reasons, were excluded from the study. Two experienced breast radiologists evaluated the breast MRIs, and three experienced breast radiologists studied the mammographies and did the ultrasound examinations and biopsies. All additional MRI findings were examined by ultrasound-guided biopsy and given a histological diagnose before it influenced the surgical method.

Results: We excluded 28 patients from the study; 10 patients choose mastectomy, 13 patients were excluded due to problems with the MRI technology, and 5 patients were excluded for other reasons. Among the remaining 117 patients, 96 were treated by breast-conserving surgery, and 21 by mastectomy. The change of operative method was induced by the preoperative breast MRI in 16 patients, because of multifocality in 11 patients, and because of a more extensive tumor than discovered by conventional diagnostic tools, in 5 patients. Five patients had a secondary mastectomy, due to histopathological findings in the lump. In one patient preoperative MRI revealed cancer in the contralateral breast, and in one patient the additional findings on MRI resulted in preoperative systemic chemotherapy. The multifocality diagnosed on the preoperative MRI was confirmed on histopathological examination of the breast in 10/11 patients. In one patient who received preoperative systemic chemotherapy, the removed breast was without sign of malignant cells on microscopic examination.

Discussion: In this prospective study preoperative breast MRI give additional information that have impact on the primary surgical treatment in 16/117 or 13.7% of the patients selected for breast-conserving surgery.

P2-08-10
Withdrawn by Author

P2-08-11
Correlation of Mammography, Ultrasound and MRI in Patients with Nipple Discharge.

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BACKGROUND: Breast cancer mainly originates in the epithelial lining of milk ducts or lobules. Once early pre-malignant or malignant changes occur in the ductal lining there is a long latent period before they manifest symptoms or are detected on imaging. Symptomatic nipple discharge (SND) is a common symptom noted in approximately 5% of patients. Although, usually benign it may be the earliest sign of cancer. The current standard evaluation of patients with nipple discharge includes clinical examination (CE), mammography (MMG) and ultrasound (US). Contrast enhanced MRI is considered the most sensitive imaging modality for the breast and reports suggest it may be of benefit in the evaluation of nipple discharge. We have combined MRI with standard evaluation in an ongoing prospective study to determine its utility in patients with SND.

PATIENTS AND METHODS: Between July 2004 and 31st May 2011, 75 women underwent surgery for SND. All were initially evaluated by CE, MMG and US following which a contrast enhanced breast MRI was requested. MRI was performed on a 1.5T MR unit using a dedicated breast coil. A STIR sequence in the coronal and sagittal planes along with an unenhanced fat-suppressed T1 W sequence was used to evaluate the ducts. The imaging findings were used to direct surgical excision. All specimens were oriented and evaluated by the pathologist by sectioning at 1 cm interval, from the ductal end to the periphery. The results of preoperative MMG, US and MRI findings were compared with pathology.

RESULTS: The incidence of cancer in this series was 29% and high risk lesions were noted in 43%. MMG and US were performed in 75 patients. MRI was requested in all 75 patients but could be performed in 64. MRI proved to be the most sensitive technique for detection of an abnormality in the breast (92%) compared to MMG 25%, and US 63%. MMG, US and MRI were suggestive of benign change in 64 (85%), 65 (87%) and 43 (67%) patients respectively, of which 14 (22%), 15 (23%) and 7 (16%) were malignant on pathology. MMG, US and MRI reported suspicious change in 11(15%), 10 (13%) and 21(33%) patients respectively of which 8 (73%), 7 (70%) and 9 (43%) patients had cancer on pathology. MRI had the highest sensitivity for malignancy (54%) compared to MMG (36%) and US (32%) but MRI had a low specificity (74%) (MMG 94%; US 94%). However, MRI had a high negative predictive value (86%) (MMG 78%; US 73%). Of the 22 patients with cancers, 12 were deemed to be normal on CE, MMG and US examination. Thus standard evaluation would have missed 54% of the cancers detected in this series.

CONCLUSIONS: Nipple discharge is associated with significant incidence of cancer and high risk changes. The majority of these are missed on standard evaluation (CE, MMG, US). MRI is the most sensitive method for the detection of an abnormality in these patients. It defines the location and extent of the abnormality and can guide surgical excision. However, MRI is unable to accurately differentiate malignant from benign lesions. Hence, excision of an identified abnormality and pathological evaluation remains essential.

P2-08-12
Additional Lesion Found in Preoperative Breast MRI: Is Routine Use Justified?

Kim J, You J, Shin H, Ahn S, Moon H-G, Cho N, Moon W-K, Han W, Noh D. Seoul National University Hospital, Seoul, Korea

Preoperative breast MRI has been increasingly performed in patients with newly diagnosed breast cancer due to its high sensitivity in assessing the extent and additional malignant foci. But due to it’s low specificity, role of routine preoperative breast MRI has become an issue. In this study we aught to analyze the characteristics of the additional lesion found in preoperative breast MRI and to evaluate the clinicopathological factors in association with likelihood of having additional malignancy. We retrospectively reviewed 2491 patients who undergone surgery due to breast cancer in Seoul National University Hospital(SNUH) between Jan 2006 and Dec 2010. Neoadjuvant chemotherapy cases, patients undergone initial sonography in other center or ones with...
prior excision were excluded and total 1068 patients were analyzed. The additional lesion was defined as the lesion not found in initial sonography and detected in preoperative breast MRI. The pathology of the additional lesion was reviewed and the association between the clinicopathologic factors and additional malignancy were evaluated. Accuracy of breast MRI was estimated regarding cancer yield, positive predictive value (PPV).

Mean age at diagnosis was 50.9 years (21 to 85 years). Overall detection rate of additional lesion was 26.2% (280 out of 1068). Mean size of the additional lesion was 9.8 mm (3-21). Additional lesions consist of 99 (35.4% of 280) C4 or higher, 174 (62.1% of 280) below C4, 7 (2.5% of 280) C0 lesions. Among them 100 patients undergone onsite surgery. 55% (55 of 100) lesions were in ipsilateral breast and 45% (45 of 100) in contralateral breast. Breast conserving surgery and mastectomy rate of the 100 onsite-operation group was 36% versus 64% and 64.6% versus 35.4% in total 1068 patients, showing no significant change of operation method of the primary cancer owing to additional lesion.

Among the 100 patients, 54 (19.3% of 280) were benign, 3 (1.1% of 280) were atypical ductal hyperplasia, 13 (4.6% of 280) were in situ carcinoma, 19 (6.8% of 280) were invasive carcinoma and 1 (3.9% of 280) were unknown. Cancer yield was 2.99% (32 out of 1068) and PPV of preoperative breast MRI was 39.0% (31 out of 82).

In univariate analysis, young age and premenopausal patients showed to have higher rate of additional cancer found in MRI (p=0.022, p=0.036). Breast density, size and LN status of the primary cancer didn’t show significance and neither the hormone receptor status with each p value 0.705, 0.381, 0.973, 0.375 respectively. Lobular carcinoma (ILC) or mixed IDC with ILC and low grade carcinoma showed significance of having additional malignancy (p=0.019, 0.022). In multivariate analysis, age, low grade carcinoma and lobular carcinoma showed independent association with p value 0.014, 0.039, 0.035 respectively (HR 0.95, 95%CI:0.94 o 0.99), (HR 0.39, 95%CI:0.16 to 0.96), (HR 5.66, 95%CI:1.13 to 28.39).

Routine preoperative breast MRI use can result in overtreatment also with delay in surgical management. In our data, younger age, low grade carcinoma, lobular carcinoma showed independent association having additional malignant foci in breast MRI. With the basis of mammography and sonography, preoperative breast MRI should only be done when additional gain is considered to overcome the flaws.

P2-08-13 Does Routine Use of Preoperative Magnetic Resonance Imaging (MRI) in Breast Cancer Influence the Outcome?
Grofe R, Kantelhardt EJ, Steer S, Spinda AK, Vetter M, Ruschke K, Thomssson C. Martin-Luther-University Halle-Wittenberg, Halle (Saale), Germany

Introduction: The role of routine preoperative breast MRI in patients diagnosed with breast cancer is still under discussion. In a milestone review, Houssami et al. (CA Cancer J Clin 2009;59:290) did not demonstrate an advantage with regard to DFS or OS. We reviewed our case series (n=793) in order to add evidence to the current discussion of whether or not preoperative MRI would influence the outcome.

Material and Methods: In our database we identified four years of diagnosis (2004-07) in which a high percentage (72%) of patients (349 of 483) with histologically confirmed invasive breast cancer received preoperative MRI. In the following years (2008/09), MRI was still done in 31% of the cases (97 of 309). All pts were treated according to national guidelines; however, patients with additional lesions in MRI were subjected to an additional MRI-guided needle biopsy or additional wire-guided excision. The median follow-up time was 31.5 months (0-81). The patients were followed clinically. In most cases local surveillance was done by mammography.

Results: In the total cohort, 20% of the patients had additional needle biopsies and/or wire-localisation due to MRI-findings. By MRI, lesions that subsequently led to an additional preoperative needle biopsy were found in 82 pts. Interestingly, only ten contralateral second cancers were found. In 357 pts, MRI was used as guidance during the operation with (n=118) or without MRI-supported wire localization (n=238). The percentage of additional MRI-needle biopsy or MRI-supported localization did not differ between invasive ductal (n=655) and lobular cancer (n=91): 10% versus 13% needle biopsies and 15% versus 20% wire localisations. The number of surgical procedures to achieve tumor-free margins did not differ between patients with or without preoperative MRI. In this series, 69 of 100 patients who received neoadjuvant chemotherapy, also had MRI-imaging. Considering the patient cohort with follow-up available (n=737), no significant difference of DFS probability was observed between pts with and without preoperative MRI.

Discussion: This retrospective analysis did not demonstrate any advantage for routine preoperative MRI with regard to local treatment and DFS probability. The observed rate of 20% additional operative procedures corresponds to similar published data (16% additional multicentric or multifocal lesions found by MRI, Solin, Breast. 2010;19:7).
HER2 expression. All other biopsies confirmed that the metastases were still positive or negative in line with the subject’s primary lesions. None of the subjects had any adverse reactions due to the investigations.

Conclusion: ABY-025 can be used safely in humans with similar excellent whole body HER2-receptor molecular imaging capabilities as have already been observed in animal models. Promising indications of in vivo HER2-receptor status discriminatory capabilities were observed.

**P2-09-02**

Predicting Response to Bevacizumab in Primary Breast Cancer Using 18F-Fluorothymidina (FLT) and 18F-Misonidazol (MISO) Positron Emission/Computed Tomography (PET/CT) as Imaging Biomarkers.

**Dominguez I, Boni V, Garcia-Vellosi MI, Lopez-Vega JM, Martinez P, Plazaola A, Llombart A, Anton A, Galve E, Alvarez IM, Hernando B, Sanchez-Gomez R, Illarramendi JJ, Morales S, De Juan A, Richter JA, Lahuerta A, Garcia-Gonzalez M, Lao Romera J, Puertolas T, Scherer S, Sabariz Luis, Garcia-Foncillas J. Clinic University of Navarra; Hospital Marques de Valdecilla; Hospital de Basurto; Oncologikoa; Hospital Arnau de Villanova de Lleida; Hospital Miguel Server; Hospital Civil de Basurto; Hospital Donostia; Hospital General Yagues de Burgos; Hospital de San Millan; Hospital de Navarra; Roche Basel; Roche Madrid**

**Background:** To investigate the hypothesis that early changes in tumor proliferation and hypoxic status induced by bevacizumab and assessed by imaging biomarkers might predict response to bevacizumab therapy.

**Methods:** 73 chemotherapy naïve, stage II-III breast cancer (BC) patients (pts) were enrolled in the training set of this phase II, single-arm, multicentric and prospective clinical trial from October 2009 until November 2010. Pts received single infusion of bevacizumab (15 mg/ kg) (C1) 3 weeks prior to the beginning of neoadjuvant chemotherapy (NAC) consisting in 4 cycles of docetaxel (60 mg/mq), doxorubicin (50 mg/mq) and bevacizumab (15 mg/ kg) every 21 days (C2-C5) following by surgery. Tumor proliferation and hypoxic status were evaluated using FLT and MISO PET/CT at baseline and 14-21 days after bevacizumab (C1). Standardized uptake values (SUV) for FLT and MISO and ratios to reference tissues, mediastinum (T/Me) or muscle (T/Mu), for MISO were calculated. Pathological response on surgical specimens was assessed according to Miller/Payne grading system. Pts with reduction in tumor cells >30% (G3-G4-G5) or <30% (G1-G2) were respectively classified as responders and non-responders. Association between pathological response, baseline and changes induced by bevacizumab (C1) in imaging biomarkers was analyzed using Mann-Whitney test. Receiver operating characteristic (ROC) curve was performed to test sensitivity and specificity of the biomarker found associated to response. Its value as independent predictor was tested in multivariate analysis using logistic regression.

**Results:** Median baseline MISO and FLT SUV values in tumors were 1.2 (range 0.69-2.39) and 2.89 (range 0.97-7.18). Significant change after C1 was observed in FLT (2.7 vs 1.8, p<0.001) but no in MISO uptake. Fifty-two (74%) pts achieved response (G3-G4-G5) whether 18 (24%) were considered as no responder (G1-G2); for FLT SUV baseline and changes after C1 in MISO SUV, T/Mu and T/Mc were all significantly associated with pathological response (p=0.057, 0.03, 0.016, 0.010). ER expression and T/Mu change remained significantly associated with response in multivariate analysis (OR=24.8, IC95% 1.8-334, p=0.01 and OR=0.95, IC 95% 0.92-0.99, p value=0.02). Decrease in MISO T/Mu uptake >20% yielded a ROC curve area of 0.7 (95% CI: 0.56 - 0.85) with 94% sensitivity and 87% specificity. Conclusion: Bevacizumab determined a marked decrease in tumor proliferation. Interestingly, a decrease greater than 20% in tumor hypoxic status after C1 and assessed by MISO was found significantly associated with pathological response suggesting a potential value of early decrease in hypoxic tumor status as predictive biomarker of response. Bevacizumab, causing normalization of the tumor microvasculature, seems to potentiate the effect of cytotoxic agents on primary BC. A validation set is warranted to confirm these findings.

**P2-09-03**


**Sella T, Sklar-Levy M, Maya C, Rozin M, Tanir A, Maira S, Libson E, Ezkay D, Hadassah Hebrew University Medical Center, Jerusalem, Israel; Chaim Sheba Medical Center; Tel Hashomer, Israel; Rabin Medical Center; Petach Tikva, Israel; Assuta Medical Center; Tel-Aviv, Israel; Kaplan Medical Center, Rehovot, Israel; Meir Medical Center, Kfar Sava, Israel; Real Imaging Ltd., Airport City, Israel**

**Introduction:** A novel prototype system performing functional three-dimensional (3D) infrared imaging (RUTH), coupled with multi-parametric computer analysis (MIRA), was evaluated for non-invasive breast cancer detection. The technique utilizes non-ionizing radiation and automatically provides risk assessment based on parameters derived from a clinically known training set.

**Materials and methods:** Following IRB approval, prospective multicenter, two-armed blinded evaluation, was performed on 102 women, which included 43 patients with biopsy proven breast cancer and 59 women with a normal (BI-RADS 1) mammogram. RUTH imaging (Real Imaging Ltd, Israel) was acquired on a continuous temporal timeline for 5 minutes during which a cold-stress test was applied for one minute. Acquired data were analyzed using multiple computed algorithms (MIRA) which were combined to develop a master algorithm for risk assessment of breast cancer. Analysis was blinded to the clinical status. Results were displayed as risk assessment scale with 100 being suspicious for breast cancer and -100 inferring normal healthy breasts.

**Results** Thirty-nine of 43 cancers (91.7%) were identified using this novel technology. The sensitivity and specificity of RUTH coupled with MIRA for detecting breast cancer were 90.7% and 67.8% respectively. The AUC defined by the ROC curve was 0.832. Twenty-seven of the 39 (70%) detected cancers were smaller than 20mm in size.

**Conclusions:** This preliminary study shows adequate performance of the novel technology examined for detecting breast cancer. Performance is comparable to the reported performance of new adjunctive breast imaging technologies such as tomosynthesis and contrast-enhanced mammography, and superior to the published sensitivity of mammography. Our results warrant further evaluation of the RUTH and MIRA technologies in detecting and characterizing breast cancer.
P2-09-04
Near Infra Red Quantum Dots as Novel Probes for Sentinel Lymph Node Biopsy.
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Background
Sentinel Lymph Node Biopsy (SLNB) is a standard procedure in breast cancer surgery. The current tracers for SLNB including the blue dye and radiocolloids have various limitations like anaphylactic reaction to the dye and exposure of radioactivity to both patients and staff. Quantum dots (QDs) are fluorescent nanoparticles (2-10nm in diameter), with unique photophysical properties like enhanced photostability and size tunable emission wavelengths, that can potentially replace the current tracers for SLNB. QDs emitting in the Near Infra Red (NIR) range of the electromagnetic spectrum can be tracked in deep tissues as biological tissues are transparent to NIR wavelengths (700-2000nm). We have developed Near Infra Red emitting Quantum Dots (NIR QDs) as alternative probes for SLNB and set up a live NIR imaging system to track them in deep tissues.

Materials and Methods
NIR emitting QDs based on CdTe:Hg were synthesized by a one pot aqueous method and characterized using Transmission Electron Microscopy (TEM), UV-Vis spectrometry and photoluminescence studies. 100µl of QDs (1mg/ml) were co-injected intradermally with blue dye into the hind legs of rat models (n=4) and compared to controls (n=4) which were injected with blue dye only. The procedure was conducted under inhalational anaesthesia using isoflurane and rats were monitored for hemodynamic instability for a period of 2hrs after injection. QDs were tracked using a live NIR imaging system including an excitation light of 630nm, emission filter of 850nm and a Hamamatsu Orca 2 UV-Vis-NIR thermoelectrically cooled CCD camera.

Results
NIR QDs had a core diameter of 7nm on TEM and emitted at 860nm upon excitation with a 630nm light source. Within 3 minutes of an intradermal injection QDs entered the lymphatic tracts. The lymphatics converged to the groin and a small surgical incision at this site revealed the underlying sentinel lymph node with minimal dissection. The rats remained hemodynamically stable throughout the duration of the procedure with no significant difference in comparison with the controls.

Conclusion
NIR emitting QDs can be used for accurate localisation of the SLN prior to surgical incision, making this an even more minimally invasive procedure and possibly an office based procedure in the future. The nanosize, surface chemistry and deep tissue visibility of these novel nanoprobes allow relentless possibilities for in vitro and in vivo molecular and cellular imaging. NIR QDs can be conjugated to biomolecules for cancer localisation, detection of micrometastasis and image guided targeted drug delivery of chemotherapeutic agents.

Further studies to investigate their in vivo biodistribution are in progress to take this technology one step closer to clinical application.

P2-09-05
Molecular Imaging of Hedgehog Induced Epithelial-Stromal Interactions.
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Introduction: Epithelial-stromal Hedgehog (HH) pathway signaling, originating from Sonic Hedgehog (SHH) ligand secreted by tumor cells, promotes breast cancer growth and metastasis. Furthermore, high tumor SHH expression is associated with poor prognosis in invasive ductal carcinoma. Studies have also shown high expression of Patched (PTCH) mRNA in SHH expressing tumors as well as cancer stem-like cells. This is in part due to a feedback loop that results in increased PTCH gene expression upon HH signaling. We sought to exploit this feedback loop by using radiolabeled derivative of SHH to identify tumors that have a high level of hedgehog signaling. These agents can potentially be used for molecular imaging of HH induced changes in PTCH expression originating from tumor expressed SHH.

Methods: Recombinant N-terminal SHH derivatives were radioiodinated with iodine-131 using the idogen method. Iodinated proteins were obtained with radiolabeling yields of 50-60 % and specific activity of 9.3E10Ci/mol. Receptor binding studies were performed on breast cancer cell lines with varying SHH expression. Studies were also conducted on cells cultured in stem cell promoting conditions (mammospheres), after exposure to ionizing radiation or treatment with the hedgehog inhibitor cyclopamine. Scatchard plots were generated using Graph pad software. Pilot studies were conducted with 110I-SHH to test the potential of imaging PTCH receptor expression in a rat model of breast cancer. Fisher rats bearing breast cancer xenografts were injected with approximately 250 microCi of 110I-SHH. Planar scintigraphy was conducted at 2, 4 and 24 hours. Studies were performed with iodinated BSA as a control for extracellular perfusion and retention. Tissue biodistribution studies were also performed using this model.

Results: Receptor binding of 110I-SHH was significantly increased in cell lines with high endogenous HH pathway activation. Receptor binding was also increased after exposure to ionizing radiation and significantly decreased following treatment with hedgehog inhibitors. Scatchard analysis revealed up to a 14 fold increase in PTCH receptor sites in mammospheres compared to the entire breast cancer population. A 10-fold increase in PTCH receptor binding sites was also observed in cells after exposure to ionizing radiation. In vivo imaging and biodistribution studies revealed significant accumulation of 110I-SHH within tumor tissue as compared to normal organs. Tumor: muscle ratios were approximately 8:1 at 4 hours, while tumor to blood and tumor to bone were 2:1 and 5:1 respectively. Significant uptake was also observed in liver tissue, as a result of protein degradation and excretion and endogenous PTCH expression.

Conclusions: These studies show that expression of the PTCH receptor is increased in cells with active HH signaling and in breast cancer mammospheres. Our findings also show that PTCH receptor expression is decreased upon treatment with HH signaling inhibitors. Preliminary imaging studies show that 110I-SHH is capable of in vivo detection of breast tumors with high hedgehog signaling. Our data suggests that radiolabeled SHH derivatives may provide a method to follow epithelial-stromal HH interactions and determine response to SHH targeted therapies.
**P2-09-06**

**Relevance of Breast Cancer Subtypes in Response Monitoring with 18F-FDG PET/CT during Neoadjuvant Chemotherapy.**

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Background – Neoadjuvant chemotherapy (NAC) is increasingly applied in stage II and III breast cancer. Response monitoring with magnetic resonance imaging (MRI) has been shown valuable, but knowledge of the breast cancer subtype is essential for correct interpretation of response assessment. (Loo et al, J Clin Oncol 29:660-6, 2011)

The aim of the present study was to evaluate the relevance of breast cancer subtype for 18F-fluorodeoxyglucose (FDG) positron emission tomography with computed tomography (PET/CT) markers for monitoring of therapy response during NAC.

Methods – Evaluation included 94 women with primary stage II or III breast cancer and measurable (quantifiable) FDG tumor uptake. FDG PET/CT scans were performed before and after six weeks of NAC using similar prone patient positioning. FDG uptake of the primary tumor was quantified using maximum standardized uptake values (SUVmax). Tumors were divided into three subtypes using immunohistochemistry: human epidermal growth factor receptor 2 (HER2) positive, estrogen receptor (ER) positive/HER2 negative and triple negative. Tumor response was assessed as presence of residual tumor in the surgery specimen (no response or partial response) or absence thereof (near complete or complete response). Multivariate regression analysis and receiver operating characteristic (ROC) analyses were employed to determine significant associations.

Results – A (near) complete response at pathology was observed in 16 (73%) of 22 HER2 positive tumors, 5 (12%) of 43 ER positive/HER2 negative tumors and 20 (69%) of 29 triple negative tumors. In the multivariate regression analysis for the whole group, (near) complete response in the surgery specimen was significantly associated with relative reduction of SUVmax of the tumor between both scans and breast cancer subtype (area under the curve of the ROC curve 0.88 [95% confidence interval 0.81-0.95], p<0.001); no significant associations were found for FDG uptake at baseline and age. In a subgroup analysis of breast cancer subtype, a significant association was found between pathologic response and relative reduction of SUVmax for ER positive/HER2 negative and triple negative tumors (p=0.012 and p<0.001, respectively), but not for HER2 positive tumors (p=0.151).

Conclusion – Knowledge of the breast cancer subtype appears relevant for the assessment of response to NAC with FDG PET/CT. Response monitoring with FDG PET/CT may predict a pathological response adequately in ER positive/HER2 negative and triple negative tumors, but seems less accurate in HER2 positive tumors. The reasons for these differences need to be elucidated in further investigations.

Disclosure – This study was performed within the framework of CTMM, the Center for Translational Molecular Medicine (www.ctmm.nl), project Breast CARE (grant 030-104).

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**P2-09-07**

**Metabolic Response by FDG-PET in Patients (pts) Receiving Trastuzumab (T) and Lapatinib (L) for HER2+ Metastatic Breast Cancer (MBC): Correlative Analysis of TBCRC 003.**

Yap JT, Locascio T, Najita JS, Mayer IA, Hobday TJ, Falkson CI, Dees EC, Gelman RS, Rimawi MF, Nanda R, Berkovic J, Franchetti Y, Wolf AC, Winer EP, Lin NU, Van den Abbeele AD. Dana-Farber Cancer Institute, Boston, MA; Vanderbilt-Ingram Cancer Center, Nashville, TN; Mayo Clinic, Rochester, MN; University of Alabama, Birmingham, AL; University of North Carolina at Chapel Hill, Chapel Hill, NC; Baylor College of Medicine, Houston, TX; University of Chicago, Chicago, IL; Johns Hopkins Kimmel Cancer Center, Baltimore, MD

Background: We evaluated the safety and efficacy of L+T in pts with 0-2 prior lines of chemotherapy (CT) for HER2+ MBC. In the context of this phase II trial, we evaluated metabolic response by FDG-PET and explored the relationship between metabolic response and clinical outcomes.

Methods: Pts with measurable, HER2+ MBC were eligible. Cohort 1: No prior T, L, or CT + T for MBC, and ≥1 yr from adjuvant T, if received. Cohort 2: 1-2 prior lines of CT for MBC, including T, or relapse within 1 yr of adjuvant T. Pts received L 1,000 mg QD + T (2 mg/kg weekly or 6 mg/kg Q3W). Staging studies were done with CT or MRI at baseline (BL) and every 2 cycles (1 cycle=4 weeks [wks]). Objective response was assessed by local investigator according to RECIST 1.0. FDG-PET/CT was performed at BL, Wk 1, and Wk 8 per NCI guidelines. Central quality assurance, review, and analysis were performed on FDG-PET studies. Up to 5 target lesions were identified on BL FDG-PET images based on hypermetabolic uptake. Percent change in the summed maximum standardized uptake value (SUVmax) was calculated at Wk 1 or Wk 8, compared to BL. Metabolic response was assessed according to EORTC criteria for % change in SUVmax (progressive disease [PD]: ≥25% increase; partial response [PR]: ≥25% decrease; stable disease [SD]: <25% change). Metabolic response at Wk 1 was compared to Wk 8 as well as to clinical outcome, including objective response, clinical benefit, and progression-free survival (PFS).

Results: 87 pts were registered to the study. Of these, one pt did not begin protocol therapy and one pt did not have MBC on further testing, and are not included. 81/85 pts had FDG-PET data at Wk 1; 75/85 had data at Wk 8. Metabolic PR at Wk 1 was observed in 28/39 (72%) pts in Cohort 1 and 20/42 (48%) pts in Cohort 2. Metabolic PR at Wk 8 was observed in 27/34 (79%) pts in Cohort 1 and 18/41 (44%) pts in Cohort 2. Wk 1 and Wk 8 metabolic responses were similar. In cohort 1, 18/28 (64%) pts who achieved Wk 1 metabolic PR had clinical benefit; 5/22 (23%) who achieved Wk 1 metabolic SD had clinical benefit. In cohort 2, 9/20 (45%) pts who achieved Wk 1 metabolic PR had clinical benefit; 5/22 (23%) who achieved Wk 1 metabolic SD had clinical benefit. In cohort 2, 9/20 (45%) pts who achieved Wk 1 metabolic PR had clinical benefit; 5/22 (23%) who achieved Wk 1 metabolic SD had clinical benefit. In exploratory analysis of progression-free survival (PFS) showed that pts in Cohort 1 who achieved Wk 1 metabolic PR experienced a median PFS of 9.1 months (95% CI 7.1-11.0); for pts with metabolic SD, median PFS was 1.6 mos (95% CI 0.8-5.5). For pts in Cohort 2, Wk 1 metabolic PR was associated with median PFS of 6.5 mos (95% CI 3.3-7.8), whereas for pts with metabolic SD, median PFS was 3.7 mos (95% CI 1.8-5.5).

Conclusions: L+T is associated with a high rate of early and sustained metabolic response by FDG-PET. Exploratory analyses suggest that metabolic PR may be associated with clinical benefit and longer PFS.
P2-09-08
FDG-PET in the Staging of the Axilla in Women with Breast Cancer Treated with Primary Chemotherapy.

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Background: FDG-PET has proven to be useful during the initial staging of several tumor types. The aim of the present study was to assess the accuracy of this test in the assessment of initial axillary status in patients referred to treatment with induction chemotherapy.

Material and Methods: Women with stage II-IIIA (tumors measuring ≥3cm or positive-staged axillary involvement) to be treated with neoadjuvant Docetaxel-Doxorubicin-Cyclophosphamide (TAC) scheme were included. In every patient a physical exam, MRI, ultrasound-guided FNAB and FDG-PET were performed prior starting chemotherapy. The criteria to consider the axilla to be initially positive were positive surgical staging or type-D pathological response (Miller-Payne) in the axilla. The ability of FDG-PET to predict pathological response in patients with breast cancer was assessed and compared with other routine procedures.

Results: 42 patients (p) were included. Median age was 45 years (30-66). 11p (26.2%) were triple-negative. 2p (4.8%) were Her-2 +. By clinical stage, 13p (30.9%) were IIA, 17p (40.4%) IIB and 12p (28.2%) IIIA. 41p (97.6%) underwent surgery after induction chemotherapy. According to surgical staging 31p (75.6%) were deemed to be N+ from the diagnosis (19p with pathologic N1-3a and 12p with type-D nodal response). PET scan positively detected deposits in the axilla in 26p (63.9%) of those patients compared to 22p (70.9%) with ultrasound-FNAB, 19p (61.3%) physical exam and 14p (45.2%) MRI.

Conclusions: In this study FDG-PET could predict axillary involvement more precisely than other routine staging procedures in these patients submitted to be treated with induction chemotherapy.

P2-09-09
Dynamic FDG PET and DCE-MRI To Assess Tumor Metabolism and Blood Flow in Response to Neoadjuvant Sunitinib and Paclitaxel Followed by AC + G-CSF in Patients with Locally-Advanced (LABC) and/or Inflammatory Breast Cancer (IBC).

Specht JM, Kurland BF, Dunnwald LK, Doot RK, Eun JK, Schubert EK, Partridge SC, Ellis GK, Gadi VK, Gralow JR, Linden HM, Rodler ET, Mankoff DA. University of Washington, Seattle, WA; Fred Hutchinson Cancer Research Center, Seattle, WA; Seattle Cancer Care Alliance, Seattle, WA

Background: Kinetic analysis of FDG PET and DCE-MRI can identify patterns of breast tumor metabolism and perfusion that predict pathologic response, relapse, and survival in patients (pts) receiving neoadjuvant chemotherapy (NC). We are enrolling pts with LABC or IBC on a phase II trial of neoadjuvant sunitinib and metronomic chemotherapy. The addition of sunitinib, a tyrosine kinase inhibitor of VEGFR1-3, PDGFR, c-KIT, to NC is hypothesized to increase rate of pathologic complete response (pCR). Assessment of FDG PET measures of glucose metabolism (Ki), glucose delivery (Kg), and MRI measures of blood flow and vascularity (peak enhancement (PE), signal enhancement ratio (SER), and volume) during NC offers the opportunity to evaluate the in vivo pharmacodynamics of sunitinib.

Methods: Pts with HER2-negative LABC or IBC participated in a companion imaging trial with [18F]-FDG PET and DCE-MRI before NC (T0), after a 1 wk run-in of sunitinib 25 mg po daily (T1), after 12 wks of paclitaxel 80 mg/m2 IV Qwk and sunitinib 25 mg po daily (T2), and prior to breast surgery (T3) after 15 wks of doxorubicin 24 mg/m2 IV Qwk, cyclophosphamide 60 mg/m2 po daily with G-CSF 5 mcg/kg SC days 2-6 each wk. FDG metabolic rate (Ki), glucose delivery (Kg), and MR indices (PE, SER, volume) were assessed. Imaging parameters were compared for groups defined by NC pathologic complete response (pCR) vs. non-pCR using a two-sample t-test.

Results: The imaging trial included 14 pts. Median age was 50 years (43-79). All had HER2-negative LABC (n=13, 93%) or IBC (n=1, 7%). Most tumors were ductal (n=12, 86%) and high grade (n=9, 64%). Seven (50%) tumors were ER negative. pCR was observed in 4/14 (29%) pts in this cohort. Changes in Ki, Kg, and MRI volume were observed between baseline (T0) and the sunitinib run-in (T1). For example, 8/14 (57%) had a decrease in Kg of ≥20%, and 3 (21%) had an increase of ≥20%. These 1 week changes did not predict subsequent response to NC. However, declines in Ki and Kg between baseline (T0) and following sunitinib and paclitaxel (T2) did predict pCR. The average change in glucose metabolism (Ki) was a 95% decline with pCR and a 68% decline otherwise (p=0.007). The average T0-T2 Kg change was 83% decline for pts with pCR and 47% decline otherwise (p=0.029). In contrast to our previous studies in LABC pts treated with NC where decline in Ki was predictive of response, decline in Ki appears to be the more robust predictor of response in this cohort. Of 11 pts with PET scans at T2 and T3, 5 showed marked increase (>20%) in Ki and 6 showed marked increase in Kg after withdrawal of sunitinib.

Conclusion: Changes in breast tumor glucose metabolism (Ki), glucose delivery (Kg), and blood flow (MR PE, SER, volume) can be detected after 1 wk of sunitinib, but are not predictive of response to NC. In the setting of anti-vascular therapy, measures of tumor metabolism (Ki) are predictive and, perhaps, more predictive of outcome than measures of glucose delivery (Kg) which may be altered by sunitinib.

*Abstracts: Poster Session 2*
P2-09-10

Giant or Windmill? 18F-FDG-PET/CT Semi-Quantitative Parameters and Biological Prognostic Parameters in Women with Breast Cancer Treated with Neoadjuvant Chemotherapy: A Multicentric Study in La Mancha.

del Mar Muñoz-Sánchez M, García-Vicente A, Ortega-Ruipérez C, Palomar-Muñoz A, Molina-Garrido MJ, Olaverri-Hernández A, Santiago-Crespo JA, Martín-Ordoñez F, Val-Pérez E, Cordero-Garcia JM, Chacón JJ, Fernández-Aramburu A, Espinosa J, Viana A, Sorianó-Castrejón A. Hospital General Virgen de la Luz, Cuenca, Spain; Hospital General Universitario de Ciudad Real, Ciudad Real, Spain; Complejo Hospitalario de Toledo, Toledo, Spain; Complejo Hospitalario Universitario de Albacete, Albacete, Spain; Hospital General Ntra Sª del Prado, Talavera, Toledo, Spain

Background: To analyze the possible predictive- prognostic value of 18F-FDG-PET/CT to evaluate response to primary preoperative neoadjuvant (NA) chemotherapy (CT) in women with breast cancer (BC) a multicenter study is being conducted in most Hospitals in Castilla – La Mancha (central Spain). As per protocol a PET/CT study has to be conducted prior to 1st cycle, after 2nd cycle and at the end of induction CT. We report here the initial results with the correlation between the PET/CT results and pathological-radiological-clinical findings prior to NA CT.

Aim: To analyze the correlation between 18F-FDG uptake assessed by PET/CT and histopathological and immunohistochemical prognostic factors in BC prior to NA CT.

Material and methods: 68 women diagnosed of BC (36 with locally advanced BC) were prospectively evaluated. PET/CT was requested in the initial staging previous to NA CT. Clinical and metabolic stages were assessed according to TNM classification. All biological prognostic parameters, such as ER, PR, p53 and c-erbB-2 expression, proliferation rate (Ki-67), and grading (SBR) were determined from tissue of the primary tumour prior to NA CT. All patients underwent an 18F-FDG PET/CT with a dual-time-point acquisition performed in the early phase 1 h after FDG administration (PET-1) and in the delayed phase 3 h after FDG administration (PET-2). Both examinations were evaluated qualitatively and semiquantitatively with calculation of SUVmax values in PET-1 (SUV-1) and in PET-2 (SUV-2) and the percentage variation of the standard uptake values (retention index) between PET-1 and PET-2. Metabolic, clinical and biological parameters were correlated. Student, ANOVA and Bonferroni tests were used to compare the means, chi-score to compare proportions and Pearson correlation to compare two quantitative variables.

Results: A positive relationship was found between the SUVmax, tumour size, clinical and metabolic stages. SUV-1 and SUV-2 values showed significant statistical correlation (p<0.05) with PET stage and tumour size assessed by PET. On the contrary, the retention index showed relation with clinical stage (p<0.05). About the biological parameters, retention index showed the best results with positive and significant relation (p<0.05) with histological grade, ER status, Ki-67 and c-erbB-2 expression. Isolated SUV values only showed significant relation to Ki-67 expression.

Conclusion: The retention index showed the best correlation with biological and clinical parameters compared to isolated SUVmax values. It may be useful as a predictive marker of tumor biological behavior. Nevertheless these are only the results of PET/CT prior to NA CT, with longer follow up we hopefully will be able to look at the correlation between PET/CT and radiological, clinical and pathological response after NA CT.

P2-09-11

Role of 18FDG-PET/CT in the Staging of Large Primary Operable Breast Cancer.


Background: Prospective evaluation of the role of 18FDG-PET/CT in patients with large primary operable breast cancer.

Material and Methods: During 56 months, consecutive patients with large (>2cm) breast cancer and clinical stage IIA/IIB/IIIA (based on clinical examination, mammography, breast MRI and ultrasonography) underwent 18FDG-PET/CT. The nuclear physician was blind to the results of any other procedure (bone scan, chest X-ray, liver ultrasound, or thoraco-abdominal CT scan).

Results: Out of the 131 examined patients, 36 had clinical stage IIA (34 T2 N0, 2 T1 N1), 48 stage IIB (20 T3 N0, 28 T2 N1), and 47 stage IIIA (29 T3 N1, 9 T2 N2, 9 T3 N2). 18FDG-PET/CT modified staging for 5.6% of stage IIA patients, for 14.6% of stage IIB patients, and for 27.6% of stage IIIA patients. However, within stage IIIA, the yield was specifically high among the 18 patients with N2 disease (56% stage modification). When considering stage IIB and primary operable IIIA (T3 N1) together, the yield of 18FDG-PET/CT was 13% (10/77); extra-axillary regional lymph nodes were detected in 5 and distant metastases in 7 patients. In this series, 18FDG-PET/CT outperformed bone scan with only 1 misclassification versus 8 for bone scan (p=0.036).

Discussion: 18FDG-PET/CT provided useful information in 13% of patients with T3 N0 / T2 N1 / T3 N1 disease. The yield was more modest in patients with T2 N0 disease. The very high yield in the case of lymph nodes classified N2 demonstrates that stage IIIA comprises two quite distinct groups of patients.

P2-09-12

High Resolution Diffusion MRI Characterizes Tumor Stromal Boundaries.

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Background: Studies of 3D cell cultures have shown that mammary epithelial cell growth and morphogenesis is regulated by extracellular matrix (ECM) stiffness, linking ECM stiffening to malignant transformation. Tumors are consistently stiffer than normal adjacent tissue, and matrix stiffening is caused by ECM cross-linking and increased deposition of collagen. Some evidence suggests that collagen orientation at tumor boundaries can promote tumor metastasis. Measuring the stiffness of the tumor boundaries and adjacent stromal tissue may give additional information 1) about tumor microenvironment and 2) to guide treatment. Diffusion-weighted imaging (DWI) MRI measures the mobility of water in tissue and may be sensitive to this phenomenon.

Material and Methods: MRI data was collected on patients with locally advance breast cancer enrolled in an IRB-approved study at UCSF and signed informed consent. In addition to a standard dynamic contrast enhanced (DCE) MRI, a high-resolution diffusion-weighted image (HR DWI) was acquired with an echo planar imaging sequence and the following parameters: TR/TE=4000/64.8 ms, b=0,600, FOV=70x140mm, matrix=28x64, and voxel size=0.55x0.55x0.4mm. Apparent diffusion coefficient (ADC) maps were created. HR DWI images were segmented into tumor and non-enhancing, surrounding stromal tissue. A proximity mapping method was used to measure ADC values at the inner edge of tumor and at increasing distances.
from the tumor boundary on HR DWI. The mean was calculated for the voxels in 1 mm increments, starting at 5 mm into the tumor (-5 mm) and ending at 2.5 cm away from the tumor (25 mm).

Results: The average of the changes per 1 mm shell was largest for the transition of the tumor boundary (Table 1). In Table 1, the -5 to 0 mm, 0 to 5 mm, and 5 to 25 mm columns represent inside the tumor, tumor boundary, and outside the tumor, respectively. In general, ADC values were consistently lower inside the tumor than outside. The greatest changes per 1 mm shell was seen in the transition from inside to outside tumor, although the values varied among tumor types. Each of the three cases analyzed had different patterns of ADC values.

Discussion: These preliminary studies show that water mobility measurements change at the tumor boundary, with different patterns observed among individual patients. We are further investigating the influence of density and tumor margin morphology on these ADC measurements.

### P2-09-13
**The Value of FDG PET/CT in Screening Detected Breast Cancer Patients.**

Koo HR, Moon WK, Cho N, Chang JM, Kang KW, Yi A. Seoul National University Hospital, Seoul, Korea

Background: To evaluate the diagnostic value of FDG PET/CT for initial staging of screening detected breast cancer.

Methods: Between January 2008 and June 2010, a total of 77 women (mean age 54 years, range 31-77 years) with screening detected primary breast cancer (mean invasive tumor size 1.65cm, range 1-70mm) underwent whole body fluorine-18 fluorodeoxyglucose (FDG) PET/CT for initial staging and were included in this retrospective study. Two patients had bilateral breast cancer. The sensitivity of FDG PET/CT for the detection of primary tumor and the sensitivity, specificity, PPV and NPV for the detection of axillary lymph node metastases were determined. Systemic staging with whole body FDG PET/CT was also performed. For analysis of diagnostic performance of FDG PET/CT, quantitative measurement of the maximum standardized uptake value (SUVMmax) criteria 1.0 was used. The final histopathology following surgery served as the gold standard.

Results: The primary tumor was FDG PET/CT positive in 65 of 79 lesions (82%). Depending on the tumor size, there was a variation in diagnostic sensitivity (63% in ≤1cm tumor, n=19 vs. 88% in >1cm tumor, n=60) and the uptake of FDG was significantly higher in >1cm tumor than in ≤1 cm tumor (mean SUVMmax 2.85 vs. 1.11, p<0.05). The uptake of FDG was significantly higher in ductal carcinomas compared to lobular carcinomas (median SUVMmax 2.0, n=72 vs. 1.3, n=7, p<0.05). Of the 77 patients included in this study, 16 patients were found to have axillary node metastasis. The sensitivity, specificity, PPV and NPV of FDG PET/CT for detection of LN metastasis were 63% (10/16), 89% (54/61), 59% (10/17) and 90% (54/60), respectively. FDG PET/CT showed distant uptake in 9 patients and 8 of 9 were false positive results. 4 lesions were confirmed histopathologically as benign and 4 lesions were evaluated with radiologic methods. One of nine was true positive result. Distant involvement was skeletal and visible on the conventional bone scintigraphy. The patient staged as cT1N3M1.

Conclusion. FDG PET/CT has limited value for the initial staging of screening detected breast cancer patients. Considering high costs, radiation exposure and false positivity, FDG PET-CT is not recommended for the preoperative evaluation of screening detected breast cancer patients.

### P2-09-14
**Evaluation of Angiogenesis Using Synchrotron Radiation in Xenograft Mouse Models of Breast Cancer.**

Gu S-M, Zhang X, Li R-M, Jin W, Shen Z-Z, Shao Z-M, Xu X-M, Wu J. Cancer Hospital/Institute, Fudan University, Shanghai, China; Shanghai Jiao Tong University, Shanghai, China

Visualization of microvasculatures is an important step in understanding the mechanism of early vessel disorders in breast cancer and developing effective therapeutic strategies. However, quantitative evaluation of breast cancer angiogenesis using immunohistochemistry has been limited in generating detailed and reproducible data. To analyze the diversification of angiogenesis in the development of tumor growth and evaluate anti-vascular effects of Avastin, a new method of microvascular imaging was introduced in this paper. We used New X-ray microangiography and third-generation synchrotron radiation-based micro-computed tomography (SR micro-CT) to investigate the structures and density of microvessels at each stage of xenograft mouse models that were created by inoculation with MDA-MB-231HM cells (one breast cancer cell line with high metastasis potential). Barium Sulfate Nanoparticles, was used as a blood vessels contrast agent through left cardiac ventricle. Three dimensional structures of microvessels were displayed with a high spatial image resolution at around 20-30 μm. The density of microvessels was significantly reduced from the third week in mouse xenograft models. This observation with SR micro-CT was consistent with the results analyzed by immunohistochemical techniques. In addition, human breast cancer–bearing mice were treated site-specifically with humanized monoclonal antibody (Avastin) that targets all isoforms of VEGF-A. Tumor volume and the density of angiogenesis were significantly reduced in xenograft mouse models of breast cancer treated with avastin compared with untreated mice as assessed by SR micro-CT. Specifically, the density of smaller vessels (di<50μm) was decreased significantly while the density of larger vessels was little changed in the Avastin-treated mice. Therefore, synchrotron radiation technique is a novel method for investigating breast cancer angiogenesis, and this might be considered as an additional complementary tool for more precise quantification of angiogenesis.

### P2-09-15
**Functional Measurements of Tumor Response during Neoadjuvant Chemotherapy Infusion and Early during Treatment Using Diffuse Optical Spectroscopic Imaging.**


Background: Non-invasive markers of neoadjuvant chemotherapy response early during treatment would provide physicians a valuable tool to make evidence-based changes to treatment strategies. In a retrospective study of 23 patients presented at SABCS 2011 we demonstrated that Diffuse Optical Spectroscopic Imaging (DOSI) can discriminate non-responding from responding subjects on the first day after the start therapy based on the presence or absence of an oxyhemoglobin flare. Here we present results of an ongoing prospective study designed to confirm the predictive nature and
biological origins of oxyhemoglobin flare and to determine if similar functional changes occur at even earlier timepoints such as during infusion.

Methods: DOSI was used to measure hemodynamic and metabolic information from tumors and surrounding normal tissue in patients prior to neoadjuvant chemotherapy, during chemotherapy infusion, and daily for the first week of treatment. DOSI uses temporally modulated near-infrared light to determine absolute tissue concentrations of oxyhemoglobin, deoxyhemoglobin, water and lipid content and requires no exogenous contrast agent. Measurements are made using a simple handheld probe placed on the skin. Blood samples were also collected at baseline and daily for seven days after the first infusion and tested for a panel inflammatory cytokines. Patients received paclitaxel + carboplatin + bevacizumab. Overall response to therapy was determined by the decrease in anatomic tumor size.

Results: To date three subjects have been measured, two of which have completed neoadjuvant chemotherapy and undergone surgery. One subject was a pathologic complete responder (pCR) and the other a non-responder (NR). In both the pCR subject and the subject still undergoing therapy there were significant functional changes measured in the tumor during infusion. A decrease in oxyhemoglobin and oxygen saturation occurred after paclitaxel infusion (saturation changes: -4.8% and -9.7% for each subject respectively), followed by an increase in these quantities after carboplatin infusion (saturation: 3.8% and 5.9% respectively). In the NR subject both oxyhemoglobin and oxygen saturation had only small fluctuations during infusion (saturation: 1.1%). Oxyhemoglobin flare was also observed 24 hours after infusion for each of the patients. Plasma levels of several inflammatory cytokines including G-CSF, MIP-1α, and C-reactive protein increased 24 hours after infusion for the first two subjects and subsequently decreased at 48 hours.

Discussion: We have confirmed the presence of oxyhemoglobin flare on day one after infusion in this small prospective patient cohort. Increased plasma levels of inflammatory cytokines were correlated in time with the presence of oxyhemoglobin flare suggesting a possible link between optical measurements and inflammatory processes induced by chemotherapy. Additionally, we report for the first time significant changes in tumoral oxyhemoglobin and oxygen saturation during chemotherapy infusion. These changes may be of prognostic significance. DOSI allows functional measurements of tumor response at timepoints unachievable with current functional medical imaging modalities.

P2-10-01
Extracellular Matrix Stiffness and Mammographic Density in the Human Breast.
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Introduction: Mammographic density (MD) is associated with greater risk to malignancy. MD is also correlated to high collagen content in the extra cellular matrix (ECM). Data from our group and others have highlighted the importance of mechanical cues from the ECM in breast tissue homeostasis and tumor progression to invasion [1; 2; reviewed in 3]. Whether the stiffness of the ECM could also initiate breast cancer and if so how remains unknown. Because elevated collagen levels increases ECM stiffness, we hypothesize that MD increases breast cancer risk because the ECM is stiffer.

Materials and Methods: We studied breast tissues obtained through prophylactic mastectomy from women with low (BIURADS 1) versus high MD (BIURADS 4). From each surgically excised breast, samples of 0.5cm x 0.5cm x 1cm dimension were removed from the retroareolar region and from 4 peripheral quadrants. Sample sections were subjected to biophysical, morphological and biochemical analysis. Biophysical analysis included the application of Atomic Force Microscopy to obtain an extensive force map of distinct anatomical regions of the ECM associated with the intra-lobular and inter-lobular ECM. Topological analysis of ECM architecture was performed using two photons and SIM-POL imaging coupled with picrosirius staining, polarized light imaging and image quantification. Biochemical and morphological analysis consisted of immunohistochemistry for markers that detect mechano-signaling in the epithelium and stromal fibroblasts, and H&E to visualize cellular and ECM organization.

Results and Discussion: We found that the intra-lobular ECM associated with the terminal end-buds in the breast contained anisotropic relaxed collagen fibrils and was very compliant. By contrast, the inter-lobular ECM of the breast contained oriented collagen fibrils and was relatively stiffer. Notably, the ECM associated with the retroareolar region, which is typically detected as very dense using mammographic imaging, contained oriented collagen fibrils, and was significantly stiffer than the ECM associated with the peripheral quadrants. Intriguingly, preliminary data suggested that the ECM associated with the terminal end-buds in the upper outer quadrant showed a trend towards greater stiffness in women with high MD (BIURADS 4) than low MD (BIURADS 1). Although, it is tempting to speculate that ECM stiffness could enhance risk to malignancy, further sample analysis is now necessary.

Conclusions:
· In the human breast there is anatomical heterogeneity with respect to ECM organization and mechanical properties.
· High MD appears to reflect elevated ECM stiffness.
· The intra-lobular ECM is considerably stiffer in the upper outer quadrant than in the other peripheral regions of the breast.
· Atomic Force Microscopy is a tractable method to monitor ECM stiffness and mechanical heterogeneity in the human breast.

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References:
1. Paszek et al., Cancer, Cell 8:241-254, 2005
2. Levental et al., Cell, 139: 891-906 2009

P2-10-02
Assessment of Tumor Response to Neoadjuvant Chemotherapy Using Ultrasound-Guided Near Infrared Light.
Zhu Q, DeFusco P, Tannenbaum S, Ricci A, Cronin E, Hegde P, Kane M, Tavakoli B, Xu Y. University of Connecticut, Storrs, CT; Hartford Hospital, Hartford, CT; University of Connecticut Health Center, Farmington, CT

Background: Neoadjuvant chemotherapy has been increasingly used in treating breast cancers. Because breast cancer is a heterogeneous disease, it is important to effectively monitor the tumor response to assist in tailoring treatments to response. In our early study, we have
introduced a novel ultrasound (US)-guided diffused light imaging in the near infrared (NIR) spectrum to monitor tumor vascular changes which correlated to tumor response. The objectives of this study are: (a) to validate the initial findings with a larger patient pool, and (b) to assess vascular changes at every treatment cycle and to correlate early vascular changes with the tumor pathological response.

**Methods:** 33 patients who underwent neoadjuvant treatment were recruited from Dec. 2007 to May 2011 and their tumor vascular content was assessed with a combined imager consisting of a commercial US system coupled to a NIR imager. Patients were imaged before their treatment, at the end of each treatment cycle and before their surgery. The co-registered US was used to localize the tumor and the NIR imager was used to map the tumor vascular distribution which was assessed based on a percentage total hemoglobin (%Hb) concentration normalized to the pre-treatment level. 18 patients were treated with AC followed by Taxol. This group of patients was monitored every two weeks at the end of each treatment. The remaining patients were on 3-week cycles of chemotherapy and monitored every 3 weeks. 6 patients were treated with TC without Adriamycin (TC), or with Adriamycin (TAC). 2 HER2 positive patients were treated with TC and Herceptin (TCH); and 3 patients were treated with AC/Bevacizumab. Pathologic response was graded based on Miller and Payne system as grade 1: non-responders (A); grades 2 and 3: partial responders (B); 4: near-complete and 5: complete responders (C).

**Results:** In the AC/Taxol group (n=18), there were 5 responders (C), 9 partial (B) and 4 non-responders (A). The statistical significance based on %Hb between groups A and C was achieved at the end of cycle 5 and the rest of the treatment cycles (p<0.05), however, the statistical significance between A and B was only obtained at end of cycle 5 (p<0.05) and not maintained for cycles 6-8. The statistical significance between B and C was only achieved at the end the treatment (p<0.05). For the TC, TAC and TCH group (n=12), there were 6 responders(C) and 6 partial responders (B). The statistical significance between these two groups was achieved at the end of cycle 3 and the rest of the treatment cycles (p<0.05). For the 3 patients who were treated with AC/Bevacizumab, 2 patients achieved complete response and one partial with grade 2. The complete responders had more than 50% reduction in %Hb at the end of cycles 3-4; while the partial one showed only 10-15% reduction during the entire treatment course.

**Discussion:** Our findings indicate that tumor vascular changes assessed by %Hb can be used to predict the tumor pathological response. This is a powerful tool to help predict responsiveness to therapy. Interestingly, dramatic and early responses were noted in the patients who received the biologic agents (bevacizumab and herceptin) and this may be very valuable in following responses using these agents.

**P2-10-04**

**3D Mapping of Total Choline in Human Breast Cancer Using High-Speed MR Spectroscopic Imaging at 3T: A Feasibility Study.**

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**Purpose:** To assess the feasibility of quantitative high-speed MR spectroscopic imaging (MRSI) of total Choline (tCho) as an adjunct to dynamic-contrast enhanced (DCE) MRI to improve lesion characterization and monitor treatment response in patients undergoing neoadjuvant chemotherapy.

**Method and Materials:** Seven patients with biopsy-confirmed, infiltrating ductal carcinoma were studied using a clinical
3T MR scanner (Siemens Medical Solutions, Erlangen, Germany) equipped with 8- and 16-channel breast array (Hologic Inc., Bedford, MA). Measurements were performed using PRESS prelocalized 3D Proton-Echo-Planar-Spectroscopic-Imaging (PEPSI) using TR/TE=2000ms/135ms, matrix size up to 32×128, voxel size=1,cc, and total acquisition time of 10 minutes (including water reference scan). Additional data were collected at TE 60 ms to enhance sensitivity for detecting tCho and J-coupled resonances. TE-averaging (8 steps, ATE: 2.5 ms) was employed to minimize gradient sideband artifacts. Quantification of tCho in reference to tissue water was performed using spectral fitting and relaxation correction.

RESULTS: Strongly elevated tCho with maximum concentration ranging from 0.3 to 4.1 mmol/kg was measured in five patients with single and multi-centric enhancing lesions larger than 2 cm volume (Table 1). The measured tCho concentration in Grade 3 tumors was higher than in lower grade untreated and treated tumors. Strong decreases in tCho concentration were measured in 2 patients undergoing neoadjuvant therapy in a follow-up scan. At short TE an additional resonance was detected that was elevated in enhancing lesions and tentatively assigned to Taurine. Two patients had lesions smaller than 2 cm with surgical clips in which tCho was not detectable due to line broadening. MRSI data sets were preferentially collected before contrast injection, since it increased spectral line width by up to 50%.

CONCLUSION: This study demonstrates feasibility of quantitatively mapping tCho in invasive breast carcinoma using high-speed MRSI. The long-term goals are to utilize high-speed MRSI as an early predictor of treatment failure in women undergoing neoadjuvant therapy (i.e. chemotherapy, endocrine therapy or biologic therapy) for breast cancer and to develop an improved screening protocol for high-risk patients.

Grant support: 1RC1EB010617-01

| TABLE 1: Clinical status and tCho concentration |

<table>
<thead>
<tr>
<th>Patient</th>
<th>Tumor Grade - Tumor Stage</th>
<th>Lesion size [mm] - ER, PR &amp; HER2 Status</th>
<th>MRSI scan 1</th>
<th>MRSI scan 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2 - T4N1M0</td>
<td>5.0 - ER+ / PR- / HER2+</td>
<td>1. Taxol +</td>
<td>0.43</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Carbo +</td>
<td>24 weeks</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Trastuzumab</td>
<td>Duration</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(HER2+ 2010)</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Herceptin +</td>
<td>NS</td>
</tr>
<tr>
<td>2</td>
<td>2 - T4N1M0</td>
<td>5.0 - ER+ / PR- / HER2+</td>
<td>1. Taxol +</td>
<td>14 weeks</td>
</tr>
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</tr>
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<td></td>
<td></td>
<td>None</td>
<td>NS</td>
</tr>
<tr>
<td>3</td>
<td>1 - T1N0M0</td>
<td>5.0 - ER+ / PR+ / HER2+</td>
<td>1. Taxol +</td>
<td>4 days</td>
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<td></td>
<td></td>
<td></td>
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<td>0.7</td>
</tr>
<tr>
<td>4</td>
<td>1 - T2N1M0</td>
<td>5.0 - ER+ / PR+ / HER2+</td>
<td>1. Taxol +</td>
<td>3.6</td>
</tr>
<tr>
<td>5</td>
<td>1 - T3N1M0</td>
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<td>1. Taxol +</td>
<td>3.6</td>
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<tr>
<td>6</td>
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<td>1. Taxol +</td>
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<tr>
<td>7</td>
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<td>5.0 - ER+ / PR+ / HER2+</td>
<td>1. Taxol +</td>
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</table>

PD - 20905

Cryo-Microcomputed Tomography (CT) with Synchrotron Radiation: Visualization of Human Breast Cancer Tissue and Comparison with Their Histopathologic Findings.

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Background: A synchrotron radiation (SR)-based X-ray source offers a powerful tool for diagnosis of breast disease due to the energy spectrum properties and the peculiar laminar beam geometry. The aims of this study were to estimate the visualization of the human breast cancer tissue with SR cryo-micro CT and to compare the results with histopathological examinations.

Material and Methods: The cancerous breast tissue samples were routinely fixed in 10% neutral buffered formalin, and each specimen was cut down to a cylindrical sample with 2 mm diameter and 10 mm height. Each breast cancer sample was rapidly frozen with dry ice, mounted on a computer-controlled precision stage and maintained at cryogenic temperature throughout data collection. Experiments were performed at the bending magnet beamline 7B2 of Pohang Light Source (PLS) in Accelerator Laboratory (PAL) which is a third-generation SR facility with 2.5 GeV operating energy. The white beam imaging system developed for synchrotron tomography consists of a 1 mm Si attenuator, 100 μm-thick CdWO4 scintillator and a full-frame charge-coupled device camera. The detector is placed 10 mm downstream from the sample on an optical table which can be rotated in the fan beam about a vertical axis for tomography. For tomography, images were collected at 0.18° increments through 180°.

The visual image was magnified using a 20x microscope objective and captured using a digital CCD camera. The spatial resolution determined by standard sample was about 1.5 μm. Three-dimensional volume images of the specimen were obtained by applying a filtered back-projection algorithm to the projection images using a software package OCTOPUS. Surface reconstruction and volume segmentation and rendering were performed using Amira software. After imaging the samples were split into several sections, processed and embedded in paraffin. Obtained tomography images were compared with corresponding histopathological findings in optical microscopy.

Results: A total of 1000 synchrotron tomography images were acquired from the samples and only a small number of different typical cases were selected for a detailed analysis. The correspondence between tomography images and histopathological findings were determined. Synchrotron tomography images yield high contrast from smoothly varying internal structures corresponding to information on actual structures seen at histopathological analysis.

Discussion: Since the projection of 3D anatomic information onto 2D image will complicate subtle difference in X-ray transmission and objects at different depths will be superimposed on each other, the subtle difference in subject attenuation, diffraction and contrast may not be clearly visible or completely lost. Tomography with SR has superior visualization of subject contrast, together with depth localization, so it is useful for visualizing anatomical structures in the sample. In this study, the obtained SR cryo-micro CT images of human breast cancer tissue were comparable with standard histopathologic findings. The results suggest that tomography with SR has a great potential as a diagnostic tool and also its clinical application is feasible, especially in breast imaging.

P2-10-06


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Background: Real-time virtual sonography (RVS) systems display virtual multiplanar reconstruction (MPR) images obtained from three-dimensional (3D) computed tomography (CT)–lymphography (LG), significantly improving preoperative detection of sentinel lymph nodes (SLNs). The purpose of this study was to prospectively evaluate SLN metastasis using an RVS system.

Methods: We identified SLNs in 73 clinically node-negative breast
cancer patients using an RVS system to display in real time a virtual MPR obtained from CT volume data corresponding to the same cross-sectional image from ultrasonography (US). CT volume data were obtained using our original 3DCT-LG, which accurately detects SLNs in breast cancer. We then prospectively attempted to predict metastasis to SLNs. SLN metastases were assessed by measuring the cortex thickness in the presence of a visible hilum. We defined suspected SLN metastases as SLNs with a cortex thickness of at least 2.5 mm on the basis of our preliminary data. All patients underwent SLN biopsy and SLN metastases were examined pathologically with serial 2.0-mm-thick multiple slices.

Results: Suspected SLN metastases were identified in 24 of 73 patients, and 13 of these 24 patients were pathologically positive. The remaining 49 patients displayed no suspected SLNs, and 46 of these 49 were pathologically negative. Sensitivity, specificity, positive predictive value, negative predictive value, and overall accuracy of measuring cortical thickness for predicting metastatic involvement of SLNs were 81, 81, 54, 94, and 81%, respectively.

Conclusion: If cortical thickness of the SLN is less than 2.5 mm, SLN metastasis is unlikely to be present. If cortical thickness of the SLN is at least 2.5 mm, preoperative fine-needle aspiration cytology may be recommended to verify the possibility of SLN metastasis.

Discussion:
TNBC and non-TNBC seem to have different ultrasonographic features. This can be explained by the unique pathologic profile of this breast cancer subtype. As some of the distinct ultrasound criteria of TNBC are associated with benign masses, TNBC may mimic nonmalignant lesions. Being familiar with these features can avoid false-negative classification of TNBC.

P2-10-08
Electrical Impedance Imaging Characteristics of Nodular and Edematous-Infiltrative Forms of Breast Cancer.
Korotkova M, Karpov A, Bulatov A, Frizyuk A. Clinical Hospital 9, Yaroslavl, Russian Federation

Introduction:
The purpose of this research is to establish diagnostic criteria of electrical impedance mammography, which are typical for nodular and diffuse forms of breast cancer.

Materials and Methods:
From 2008 to 2011, we examined 558 patients, 34 of whom had various forms of breast cancer (BC). All the patients were examined with electrical impedance potential mammography utilizing the electrical impedance computer mammograph MEIK, which enables to acquire images of 3-D conductivity distribution layers within mamma’s tissues up to 5 cm depth.

Group I comprised 27 women suffering from nodular forms of BC. Among them 9 women without clinical signs of the disease were put into subgroup Ia.

19 patients having significant clinical signs, such as edema, inflammation and infiltration, were put into subgroup Ib.

Group II included 6 patients with diffuse BC.

102 patients without any mammary gland disease were put into the control group.

Both visual and quantitative assessment of electrical impedance mammograms (EIM) was conducted.

Result:
Distinctions of kind were revealed between EIMs of the abovementioned patient groups.

In case of nodular BC both general and local changes were observed in EIMs.

General changes include deformation of mamma’s contour, hyperimpedance contour and displacement of inner structures.

Local changes comprise focal changes of electrical conductivity in form of an- or isoimpedance areas and hyperimpedance contour on the border of the tumor and surrounding tissue.

In EIM mammograms of 100% patients belonging to subgroup Ia only local changes of electrical conductivity were detected in a form of animpedance area presented on several scan planes in the location corresponding to the tumor (electrical conductivity index >0.95), while there were no general changes in the image. The size of lesions detected in this group was less than 1 cm.

Clinical signs of the decease were always present in group Ib patients. EIMs of these patients revealed both general and various local changes.

Visual estimation of images revealed deformation of the mammary gland contour in 50% of the cases, subcutaneous fat infiltration - in 61%, anatomic changes with displacement of internal structures in 78%, perifocal infiltration in 94%. The quantitative analysis established the following: mean electrical conductivity index of a healthy mammary gland, as a rule, exceeded the index of mean electrical conductivity of the affected one; and the index of the
mean electrical conductivity of a tumor exceeded the index of mean electrical conductivity of the affected gland almost twofold. EIMs of 100% of the patients with diffuse (edematous-infiltrative) BC revealed typical general changes of electrical conductivity: electrical conductivity of the affected mammary gland is considerably lower than that of the healthy one as well as lower than that of the age norm. This results in the intense dark EIM and significant change of electrical impedance anatomy.

Conclusions:
The revealed peculiarities of the electrical impedance images during various forms of the infiltrating BC growth correlate with the pathological-physiological phases of the tumor development. The article is illustrated with electrical impedance mammograms (EIMs) and tables.

**P2-10-09**

**Detecting Breast Cancer with Dynamic Diffuse Optical Tomographic Imaging.**

*Flexman ML, Kim HK, Lim E, Desperito E, Barbour RL, Hershman DL, Hielscher AH. Columbia University, New York, NY; State University of New York, Brooklyn, NY*

**Background:** Over the last decade diffuse optical tomography (DOT) has emerged as a novel medical imaging modality. Near-infrared light is used to non-invasively probe biological tissue, and three-dimensional (3D) maps of blood-dependent parameters can be obtained. Several studies are underway to show the clinical utility of DOT for imaging brain disease, joint disease, and breast cancer. Here we present the largest clinical study to date that uses hemodynamic effects caused by a simple breath hold to identify breast tumors using DOT.

**Methods:** We have designed and built a DOT breast imaging system that can acquire full 3D data sets within a fraction of a second. The system affords the ability to study fast hemodynamic effects in both breasts simultaneously. Using non-compressive imaging heads we measured the oxygenated and deoxygenated hemoglobin levels in the breasts of 15 patients whose mammogram showed a mass >1 cm (4 benign, 11 malignant) and 3 healthy controls. Data was acquired over the course of a 30-second breath hold and 30 seconds thereafter. In addition, a baseline measurement of 30 seconds prior to the breath hold was obtained. A multi-wavelength image reconstruction algorithm was used to create 3D maps of hemoglobin-dependent parameters (Δ[HbO2] and Δ[Hb]) in the breast every 0.58 seconds, over the course of the 90-second experiment. An image analysis algorithm identified regions of peak percentage change in [Hb] and [HbO2] in the breast and then computed the average hemoglobin levels in those regions.

**Results:** We observed an increase in the hemoglobin levels in all breasts during the breath hold. Upon resuming breathing, these hemoglobin levels returned to baseline. Tumor bearing breasts showed a statistically significant slower return to baseline than healthy breasts. In particular, we found that tumors can be detected by a substantially larger Δ[Hb] value as compared to normal tissue in images acquired 15 seconds following the end of the breath hold. In 10 of 11 patients the malignant tumor was identified using this technique, suggesting sensitivity over 90%. No regions of increased Δ[Hb] were seen in the healthy breasts, or in the breasts with benign masses. The peak percentage change in [Hb] at the 15 second post-breath hold time point was 10.0 ± 6.0% (n=11) in the malignant tumors compared to 1.4 ± 0.5% (n=3) (p=0.001) in healthy patients and 4.8 ± 1.9% in benign masses (n=4) (p=0.03).

**Discussion:** A breath hold impedes venous return to the heart, which causes pooling of blood in the breast. This is observed as an increase in [Hb] and [HbO2] using DOT. Tumor vasculature is known to be more disorganized, tortuous, and leakier than normal vasculature. Therefore, once the breath hold is released and blood is allowed to drain from the breast, blood accumulated in the tumor during the breath hold will drain more slowly than blood in healthy tissue. This study has shown that DOT allows us to visualize these hemodynamic effects and use them to detect tumors with a simple breath hold and compression-free imaging head. Future studies need to explore the detection limits and general clinical utility of this technique for screening, differentiating malignant from benign masses, and treatment monitoring.

**P2-10-10**

**A Precision Comparison of Breast Ultrasound Images between Different Time Phases by Imaging Fusion Technique Using Magnetic Position Tracking System.**

*Nakano S, Fujii K, Yorozuya K, Yoshida M, Fukutomi T, Arai O, Mitake T. Aichi Medical University, Nagakute, Aichi-gun, Japan; Hitachi Medical Corporation*

**Purpose**
We developed a real-time virtual sonography (RVS) in which real-time ultrasound (US) images and reconstructive virtual US images of the same cross-section are synchronized based on US volume data obtained on the same monitor using magnetic position tracking system (US-RVS). The purpose of this study was to evaluate the accuracy of US-RVS to compare breast US images between different time phases.

**Materials and Methods**
Between March 2010 and April 2011, US-RVS was performed in 108 consecutive patients with 112 target lesions for US (mean lesion size: 12 mm, 54 malignant and 58 benign). We used US-RVS system which consisted of sonography equipment, magnetic field generator, magnetic sensor, and workstation. To assess the accuracy of the US-RVS, we analyzed the sonographic re-identification rate of target lesions in different time phases.

**Results**
Among the 112 target lesions, 105 (94%) lesions were re-identified by US-RVS. The remaining occult 7 (6%) lesions were included one DCIS and 6 fibrocystic changes in terms of the histological type, and one mass lesion and 6 nonmass like lesions in terms of morphological feature. US-RVS enabled us to precisely compare arbitrary cross-sections of US images in any direction between different time phases independent of the operator's skill.

**Conclusion**
US-RVS, which requires no contrast medium and involves no radiation exposure, can be a useful modality for temporally tracking regions of interest using US.

**P2-11-01**

**Validation of Predictive DNA Damage Response (DDR) Assay in Luminal B and Basal-Like Breast Cancers.**

*Rodriguez AA, Mao Y, Zhao H, Wong S, Chang JC. The Methodist Research Institute, Houston, TX*

**Background:** We previously published a 69-gene DDR microarray signature that predicted for pathologic complete response (pCR) to anthracycline chemotherapy in women with basal-like breast cancer in two clinical studies. Here, we sought to validate this predictive signature in independent data sets of breast cancers, selecting for...
basal-like and luminal B subtypes. We also customized a low density qRT-PCR array for use on formalin-fixed paraffin embedded tissue that will allow for prospective validation and clinical use.

Methods: Six independent validation sets were selected with known response to DNA damaging agents, containing either anthracyclines or cisplatin. One independent data set was known to response to the microtubulin agent docetaxel (non-DNA damaging) was used. Using PAM50 genes, the tumors were first classified according to the molecular subtype. The predictive value of the 69-gene DDR assay was then correlated with known pathologic response, and the receiver operating curve determined.

We then performed qRT-PCR of these 69 genes with 5 controls, and correlated the expression of these genes with microarray RNA values in an additional 50 basal tumors. The data for individual genes was normalized, combined, and then hierarchically clustered.

Results: In the 170 basal tumors treated with DNA damaging agents, a high score predicted response in basal-like tumors, A, p<0.05. Conversely, for a non-DNA-damaging therapy, a high score significantly predicted for lack of response. B. In the 116 luminal B cancers, the DDR-assay also predicted for response to DNA damaging agents containing regimens with AUCs ranging from 0.67 to 0.80. C.

Prediction of DDR assay on Public Datasets

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>pCR</th>
<th>AUC</th>
<th>95% CI</th>
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<tr>
<td>Basal-like tumors</td>
<td></td>
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<td>- Response</td>
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<tr>
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<td>- Response</td>
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<tr>
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<td>365</td>
<td>6</td>
<td>0.67</td>
<td>0.42-0.91</td>
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</table>

N: number of patients, pCR: complete and near pathological response, AUC: area under the curve.

Microarray of the 69 DDR gene expression values was compared with Ct values by q-RTPCR, low density array for 50 paired samples. High concordance between these two platforms (48/50 samples) was observed.

Conclusion: We have identified a robust predictive DDR assay that predicts for response to the DNA damaging agents, doxorubicin, epirubicin, and cisplatin in multiple studies, but not for docetaxel therapy. Future studies are underway to test for prediction of benefit of PARP1 inhibitor in basal-like and luminal B breast cancer.

References:

P2-11-02
Brain-Derived Neurotrophic Factor Expression Is Associated with Poor Prognosis in Human Breast Cancer.

Patani N, Jiang WG, Mokbel K. The London Breast Institute, The Princess Grace Hospital, London, United Kingdom; University Department of Surgery, Cardiff University School of Medicine, Cardiff, United Kingdom; Brunei Institute of Cancer Genetics and Pharmacogenomics, Brunei University, London, United Kingdom

Background: Brain-derived neurotrophic factor (BDNF) belongs to the neurotrophin superfamily of polypeptide growth factors whose physiological roles primarily relate to the development and function of the vertebrate nervous system. However, BDNF is also expressed in non-neuronal tissues and has been implicated in breast cancer, in addition to several other human malignancies, including: neuroblastoma, myeloma, ovarian, lung, prostate, hepato-cellular, pancreatic, head and neck squamous cell carcinomas and pulmonary carcinoid tumours. Although increased neurotrophin and cognate receptor expression have been demonstrated in breast cancer, the emerging role of BDNF in tumour biology and its utility as a novel biomarker have yet to be fully elucidated. In this study, the mRNA and protein expression of BDNF are evaluated in women with primary operable breast cancer in a well annotated cohort with extended follow-up.

Methods: Breast cancer tissues (n=127) and normal/benign tissues (n=33) underwent RNA extraction and reverse transcription. Transcript levels of BDNF were determined using real-time quantitative PCR and protein expression was assessed using standardised semi-quantitative immuno-histochemical techniques. Expression levels in neoplastic tissues were compared with adjacent normal/benign samples and evaluated against conventional pathological parameters, including: tumour size, grade, nodal involvement, TNM stage, in addition to Nottingham Prognostic Index (NPI), disease free and overall survival over a 10 year follow-up period.

Results: BDNF was found to be expressed in both normal breast tissue and breast cancer specimens. Significantly greater BDNF expression was identified in neoplastic cells, compared to normal mammary epithelial cells. BDNF mRNA transcript levels were also found in breast cancers compared to normal samples (p=0.007). Increased BDNF transcript levels were found to be significantly associated with nodal positivity (p=0.047) and increased with NPI; NPI-1 vs. NPI-2 (p=0.009). Higher BDNF expression was significantly associated with local recurrence (p=0.0014), death from breast cancer (p=0.018) and poor prognosis overall (p=0.013), when compared to patients who remained disease free. Higher transcript levels were significantly associated with poorer overall survival (106 vs. 136 months, p=0.006) after a median follow up of 10 years.

Conclusion: Neurotrophins and their receptors are increasingly being implicated as novel mediators of carcinogenesis in neuronal and non-neuronal tissues. BDNF is overexpressed in breast cancer and significantly associated with adverse pathological parameters, including nodal positivity and increasing NPI. Higher BDNF expression was significantly associated with poorer clinical outcomes, including local recurrence, death, poor prognosis and reduced overall survival. The present study adds to the literature in support of the oncogenic function of BDNF in breast cancer and is the first to quantitatively evaluate expression in a large cohort. BDNF expression may offer prognostic utility as a biomarker and further mechanistic studies are warranted to explore the potential for targeted therapeutic manipulation.

P2-11-03
High Expression of CX3CL1 by Tumor Cells Correlates with a Good Prognosis and Increased Infiltrating CD8+-T Cells, Natural Killer Cells, and Dendritic Cells in Breast Carcinoma.

Lee JS, Park MH, Yoon JH. Chonnam National University Medical School, Gwangju, Republic of Korea

CX3CL1 is the only CX3C chemokine that can chemottract CD8+-T cells, natural killer (NK) cells, and dendritic cells (DCs). Although studies have reported that CX3CL1 regulates the host immune response, the roles of the CX3CL1 in breast carcinoma remain unknown. We evaluated the expression CX3CL1 in 198 surgical specimens of breast carcinoma and analyzed any association with the clinicopathological factors, including CD8+ T cells, CD56-
NK cells, and CD1a+ DCs, while also evaluating the impact on the prognosis. High CX3CL1 expression was found in 136 of 198 (68.7%) breast carcinomas. The numbers of stromal CD8+ T cells, intratumoral CD1a+ DCs, and stromal CD56+ NK cells were significantly increased in the high CX3CL1 expression group (19.3 ± 15.2, 0.3 ± 0.9 and 1.4 ± 1.7 per field, respectively) compared with the low expression group (10.2 ± 13.1, 0.0 ± 0.3 and 0.7 ± 0.8 per field, respectively). Patients with high CX3CL1 expression had a significantly better prognosis for disease-free and overall survival than those with low expression (P = 0.002 and P = 0.000, respectively). In a multivariate analysis, the CX3CL1 expression was identified as one of the independent prognostic factors for overall survival (odds ratio, 2.29; P = 0.002). These data suggest that CX3CL1 expressed in the tumor cells appears to enhance the recruitment of CD8+ T cells, NK cells, and CD1a+ DCs, thereby bringing about a better prognosis in breast carcinoma. CX3CL1 is a new prognostic biomarker and be a novel candidate for development of a more effective therapeutic strategy for breast carcinoma.

**P2-11-04**

**TMEPAI Is a Feedback Regulator of TGF-β Signaling during Breast Cancer Progression.**

Singha PK, Pandeswara S, De La Chapa JJ, Venkatachalam MA, Saikumar P. UT Health Science Center at San Antonio, San Antonio, TX

**Background:** Despite lack of hormone receptors (ER/PR) and HER2, many triple negative breast cancers (TNBC) depend on transforming growth factor beta (TGF-β) signaling activity for late growth and metastasis. Thus TGF-β signaling is a major potential target to treat TNBC patients. However, inhibition of TGF-β signaling pathway carries the risk of disturbing the tumor suppressive activity of TGF-β in normal tissues and early cancers. Hence, present study was undertaken with a goal to identify suitable markers which will enable to develop drugs that retard only the “oncogenic activity” of TGF-β while preserving its growth suppressive activities.

**Materials and Methods:** All cell lines were cultured according to the recommended standard procedures. Lentiviral mediated expression vector was used to stably knockdown endogenous TMEPAI expression. DNA transfections and luciferase assays were performed according to vendor instructions. Cell proliferation was performed according to vendor instructions. Cell proliferation was measured by quantitation of total cellular DNA. Immunoblotting and immunohistochemical analysis were performed using standard methods.

**Results:** Previously we have shown that Transmembrane prostate androgen-induced protein (TMEPAI), a TGF-β inducible gene has the potential to convert tumor suppressive TGF-β into a tumor promoter. In the present study, we show that Smad binding elements driven luciferase reporter activity, a measure of TGF-β signaling, was dramatically increased in TMEPAI knockdown cells. While wild type cancer cells showed transient raise in phosphorylation of Smad2 and Smad3 with TGF-β treatment, TMEPAI knockdown resulted in increased and sustained levels of phosphorylated Smad2 and Smad3. In normal mammary epithelial cells, continuous presence of TGF-β blocked their growth. In contrast, breast cancer cells showed a biphasic growth response (moderate inhibition up to 72h followed by strong growth stimulation) to TGF-β, while TMEPAI-deficiency blocked this response to TGF-β. Furthermore, exogenous expression of TMEPAI in normal mammary epithelial cells and TMEPAI-knockdown cells resulted in reduced TGF-β-dependent Smad activity. Additionally, we found that TMEPAI subverts tumor suppressive TGF-β dependent Smad signaling into tumor promotive non-Smad signaling through stimulation of stress activated protein kinases. Inhibition of stress kinases also reduced the growth of cancer cells similar to TMEPAI deficiency. To evaluate the translational importance of TMEPAI as predictive marker, immunohistochemical analysis on human breast cancer specimens revealed the gain of TMEPAI expressions in aggressive human breast tumor samples but not in normal human breast tissue specimens.

**Conclusions:** Our results show that TMEPAI, which subverts tumor suppressive Smad signaling, may serve as a novel prognostic and predictive marker for aggressive and TGF-β dependent of metastatic breast cancers. These studies will further provide new clues to develop an effective and decisive anti-TGF-β therapy against aggressive breast cancers without disturbing the growth suppression by TGF-β in normal tissues and early tumors.

**P2-11-05**

**Stromal Matrix Metalloproteinase-14 Expression Correlates with the Grade and Biological Behavior of Mammary Phyllodes Tumors.**

Lee JS, Park MH, Yoon JH. Chonnam National University Medical School, Gwangju, Republic of Korea

Phyllodes tumors (PTs) of the breast are rare biphasic tumors with the potential for invasion and metastatic spread. Matrix metalloproteases (MMPs) and their tissue inhibitors of metalloproteases (TIMPs) are involved in several key aspects of tumoral growth, invasion and metastasis, but little is known of their expression in PTs. The objective of this study was to assess the expression of MMPs and TIMPs in PTs and to determine their association with grade and clinical behavior of PTs. Eighty-two PTs (50 benign, 22 borderline, and 10 malignant) were studied. Automated immunohistochemical staining for MMP-1, -2, -7, -9, -11, -13, and -14 and TIMP-1, -2, and -3 was performed using tissue microarray blocks and the expression of MMPs and TIMPs was assessed in both the stromal components. There were no significant differences in the expression of stromal MMPs and TIMPs in the three groups of PTs, except for MMP-14. There was a significant increase in stromal MMP-14 expression with increasing PT grade (P < 0.01). The stromal MMP-14 expression in the borderline and malignant PTs was higher than that in benign PTs (P < 0.05 and P < 0.05, respectively). Furthermore, the expression of stromal MMP-14 was associated with a higher rate of recurrence (P < 0.05). Our results show for the first time that stromal MMP-14 expression is associated with the grade and clinical behavior of PTs of the breast.

**P2-11-06**

**PTEN Loss in Asian Triple Negative Breast Cancer Is a Frequent Event Associated with Basal Markers, Tumour Grade and Younger Age of Onset.**

Dean S, Rhodes A, Holly J, Perks C, Looi L-M, Mun KS, Taib NA, Tip CH. University of the West of England, Bristol, United Kingdom; University of Bristol, Bristol, United Kingdom; University of Malaya, Kuala Lumpur, Malaysia

Triple negative (TN) breast cancers are defined by their lack of expression of hormone receptors and HER2 and account for approximately 15% of all breast cancers. They are highly aggressive tumors for which there is currently no effective targeted therapy. PTEN is a tumor suppressor gene that shows loss or mutation in many types of cancer. PTEN functions by deactivating Akt signaling so cell growth is inhibited and apoptosis is promoted. PTEN has also been found to have an important role in DNA repair. Cancers with PTEN loss have been shown to be sensitive to PARP inhibitors that
prevent DNA repair and result in tumor cell death. In unselected breast cancer cohorts with the expected frequency of HER2 and hormone receptor positive cases, its loss appears to be a relatively rare event. Insulin-like growth factor binding protein-2 (IGFBP-2) regulates the anti-apoptotic and mitogenic effects of insulin like growth factors (IGF's) -I and -II and can also exert its own intrinsic effects on cell function; in vitro cell line models have shown that IGFBP-2 down regulates PTEN expression by interacting with the β-integrin receptor. The purpose of the current study was to investigate the extent of PTEN loss in an Asian series of TN breast cancers also to determine the association of PTEN loss with IGFBP2 expression and clinicopathological features. 122 TN breast carcinomas from the University of Malaya Medical Centre were immunohistochemically (IHC) stained for CK5/6, CK14, PTEN and IGFBP-2. The basal-like phenotype was defined as positivity for either one or both of CK5/6 and CK14, in invasive tumour cells. A modified Allred scoring system was used to assess the IGFBP-2 staining. PTEN was assessed for loss in both the cytoplasm and the nuclei; the surrounding stromal tissue and normal ducts had to be positive for the case to be assessable. Expression of basal CKs were present in the majority of cases, with 72.5% and 70.4% of TN cases staining positively for CK5/6 and CK14, respectively. Loss of nuclear and cytoplasmic IHC staining for PTEN occurred in 44.9% of TN cases. In those cases with PTEN loss, 88.4% were positive for CK5/6 compared to only 66% for cases without PTEN loss. (p=0.011). The median age of patients with tumors showing PTEN loss were 48 years old, compared with 56 years in those without PTEN loss (p=0.007). Of the cases showing PTEN loss, 35/40 (87.5%) were Grade 3 tumors; in comparison 28/46 (60.9%) of tumors without PTEN loss were Grade 3 (p=0.008). With respect to IGFBP2, 26.5% of cases were positive using the cut-off score of 6 or greater. However, there was no significant association between PTEN loss and IGFBP2 expression (p=0.542). Complete PTEN loss was a frequent event in a cohort of nearly one hundred TN breast cancers; this was significantly associated with younger age. PTEN loss can be readily and reliably assessed by an optimised IHC assay and validated PTEN antibody. The extent of PTEN loss occurring in TN breast cancers may well reflect a defect in DNA double strand repair and therefore, along with other suitable markers, may prove to be a valuable assay to identify those patients with TN breast cancers that are most likely to respond well to PARP inhibitors.

P2-11-07
Expression of Selected Predictive Markers in African American Women with Atypical Hyperplasia of the Breast.
Bandyopadhyay S, Cote M, Visscher DW, Ruterbusch J, Albashiti B, Alosh B, Frost MH, Hartmann LC, Fehmi RA. Wayne State University/KCI/DMC, Detroit, MI; Wayne State University/KCI, Detroit, MI; Mayo Clinic Cancer Center, Rochester, MN

Invasive breast carcinoma in African American (AA) women differs significantly from their Caucasian (CA) counterparts in its incidence, morphology and outcome. These tumors are more likely to be high grade, hormone receptor negative, present at a younger age and at a higher stage. Evaluation and a better understanding of precursor lesions may help delineate the mechanisms underlying the development of breast cancer in these two groups. Atypical hyperplasia (AH) in the breast has been associated with an increased risk of developing cancer (relative risk~4.0). Risk stratification of these women by identification of predictive biomarkers would be beneficial for optimal patient care. In our study we evaluated the expression of the following prognostic biomarkers: estrogen receptor (ER), Cyclooxygenase-2 (COX-2) enzyme and Ki-67 in AH in a cohort of AA women with benign breast biopsies. AA women with benign breast biopsies from years 1997-2000 were retrieved from our departmental database. Clinical and follow up data was obtained from the SEER database. The hematoxylin and eosin (H & E) slides for these cases were reviewed by 2 pathologists, who were blinded to the outcome, and those with atypia were included in this study. Paraffin blocks were retrieved for immunohistochemical (IHC) analysis and standardized scoring methods applied. A total of 1608 AA women had benign breast biopsies during the study period. We performed IHC analysis on 37 (2.3%) who were diagnosed with atypia (25 cases of atypical ductal hyperplasia (ADH) and 12 cases of atypical lobular hyperplasia (ALH)). Increased COX-2 expression was seen in 19 of 28 (67.8%) cases with AH. Of these, 13 of 19 cases (68.4%) were of ADH and 6 of 9 cases (66.7%) were of ALH. Twenty of 25 cases had a high expression of ER overall. Of these, 15 of 17 (88.2%) of the positive cases was in the ADH category and 3 of 7 (42.8%) was in the ALH group. Of 32 cases, only 3 cases showed a proliferation rate of > 2% (9.4%) with Ki-67 IHC stain. All of these cases belonged to the ADH (21) category. In summary, the majority of AH cases showed increased COX-2 expression, although no differences were observed between lobular and ductal lesions. In contrast, ADH lesions appeared to exhibit increased reactivity for ER compared to ALH. Similarly, although rare, more ADH cases showed an increased proliferation rate compared to ALH. From our data, COX-2 and ER might be of prognostic significance in AA patients with AH. Larger studies with follow up are needed to understand this disease further.

P2-11-08
Schmitt M, Kiechle M, Schwarz-Boeger U, Langer R, Nakayama S, Matsushima T, Ishihara H. Technical University of Munich, Munich, Germany; Sysmex Corporation, Kobe, Japan

[Background] C2P is an assay measuring specific activities (SA; kinase activity compensated by its protein expression) of cyclin-dependent kinases CDK1 and CDK2 by examining a small piece of fresh-frozen tumor tissue. We reported previously that the C2P risk score given by CDK1SA and CDK2SA is a potent prognostic factor in node-negative breast cancer patients. Likewise, a uPA/PAI-1 ELISA test (FEMTELLER®, American Diagnostica Inc. Stamford, CT) quantitatively determines uPA (urokinase-type plasminogen activator) and PAI-1 (plasminogen activator inhibitor type-1) antigen levels in tumor tissue extracts, to identify patients with high or low risk of disease recurrence of node-negative breast cancer patients. These cancer biomarkers are recommended by the ASCO at the highest level of evidence (LOE-1) for therapy decision making in node-negative breast cancer patients. From the biological point of view, the above two assays can be placed into two categories: tumor cell proliferation for C2P and tumor cell invasion for uPA/PAI-1. This fact led us to examine the concept of combination of the two assays to better select breast cancer patients at risk. [Results] Fifty-nine cases of frozen primary breast cancer tissues were subjected to the C2P assay in a blinded manner. Twenty-one cases (40%) were categorized into “high risk”, 7 cases (14%) into “intermediate risk”, and 24 cases (46%) into “low risk”. Seven cases were judged as “not informative". The uPA/PAI-1 results and clinical information (26: recurrent cases, 30: non-recurrent cases, 1: stage IV, 2: unknown) were

provided by the TUM tumor bank, uPA/PAI-1 risk categories of 36 cases (61%) were classified “high” and 23 cases (39%) categorized “low”. C2P and uPA/PAI-1 showed statistically significant correlation to histological grading (Pearson correlation coefficient; 0.45 and 0.40, respectively). No significant correlation was observed between C2P and uPA/PAI-1. By Kaplan-Meier analysis for disease-free survival, in cases treated with endocrine therapy only, both C2P and uPA/PAI-1 showed a reproducible trend to the respective claimed performances as prognostic factors. In the combination analysis of the two parameters, where low/low was judged as “low” and the others as “high”, 11 cases (24%) were categorized into “low” and 34 cases (76%) into “high”. The sensitivity and negative predictive value for disease recurrence were 90% (19/20) and 91% (10/11), respectively. Strong statistical significance was observed between the risk categories by the log-rank test; \( p = 0.0089 \), and also by Cox proportional hazards regression analysis; \( HR = 9.18, \ p = 0.032 \). By multivariate analysis, also including tumor size and nodal status, the CP2 uPA/PAI-1 combination evolved as a significant, statistically independent parameter (HR: 6.51, \( p < 0.01 \)). [Conclusion] In this investigative study, the significance of the combination concept was strongly suggested for early breast cancer patients treated with endocrine therapy only. Yet, prior to implementation in the clinical setting, the practical performance of the combination assay should be validated by investigating an independent patient cohort.

### P2-11-09

#### EGFR Overexpression in Triple Negative Breast Cancer (TNBC) and Its Association with the Prognosis.

Liu W, Zhang L, Ma K, Han B, Li S, Xu G, Fan Z, Liu N, Shi A. The First Hospital of Jilin University, Changchun, Jilin, China; The 208 Hospital of People's Liberation Army, Changchun, Jilin, China; The Central Hospital of Siping, Siping, Jilin, China; The Second Hospital of Jilin University, Changchun, Jilin, China; The Siping Center Hospital, Siping City, Jilin Province, China

**Objective:** The aim of this study is to investigate EGFR expression in Triple Negative Breast Cancer (TNBC), and to find the relationship between EGFR overexpression and prognosis of TNBC, further to clarify the significance of EGFR in TNBC and provide valuable information for TNBC therapy.

**Methods:** 42 triple negative breast cancer patients (staging group) and 40 HER2+ breast cancer patients (control group) who underwent surgery from January 2000 to December 2005 were analyzed. 82 cases of paraffin-embedded specimens were detected by Immunohistochemistry (IHC), fluorescence in situ hybridization (FISH), and polymerase chain reaction (PCR) to investigate the overexpression, amplification and mutation of EGFR gene. The distant-free survival (DFS) and overall survival (OS) of these patients were used to investigate the relationship between EGFR overexpression and the prognosis of TNBC.

**Results:** 34 (43.9%) EGFR overexpression was observed in all cases, while gene amplification was only found in 7 (9.1%) cases. No EGFR gene mutation was found in all cases. Overexpression of EGFR occurring in 57.1% patients in TNBC group and 25.0% patients in HER2 group, and we didn’t find any correlation between EGFR overexpression and clinicopathology. 50 (61.0%) patients relapsed (TNBC 28, HER2 22) and 27 (32.9%) patients died (TNBC 18, HER2 9) were observed during the more than 5 years follow-up. The 5-year DFS was 57.1% and 77.5% respectively, the 5-year OS was 71.4% and 87.5% in TNBC and HER2 groups. In TNBC group, the survival of the EGFR-overexpressing group patients was significantly lower than that of the non-EGFR-overexpressing group patients (\( p = 0.018 \) for DFS, \( p = 0.026 \) for OS); In HER2 group, no statistical difference was found (\( p = 0.079 \) for DFS, \( p = 0.055 \) for OS).

**Conclusions:** This study showed that EGFR overexpression increased significantly in TNBC patients, which was no correlation with their clinicopathological data. EGFR gene amplification was much less frequent than its overexpression. It suggested that EGFR gene amplification may not be the unique mechanism of EGFR overexpression in TNBC. There may be other possible mechanisms and pathways that cause EGFR overexpression. In addition, it may suggest that gefitinib therapy is useless in TNBC patients because we did not find any mutations in the tested exons of TNBC. EGFR overexpression may associate with a poor outcome of TNBC patients which suggest it could be a significant prognostic factor for TNBC patients. EGFR may play important role for molecular-targeting therapy of TNBC.

### P2-11-10

#### Low Toll-Like Receptor 9 Expression Is Required for the Aggressive Behavior of Triple Negative Breast Cancer Cells in Hypoxia.

Selander KS, Tuomela J, Sandholm J, Karihtala P, Pressey C, Ilvesaro J, Vuopala K, Kauppila JH, Kauppila S, Harris KW, Graves D, Jukkola-Vuorinen A. University of Alabama at Birmingham, Birmingham, AL; Oulu University Hospital, Oulu, Finland; University of Oulu, Oulu, Finland

Toll-like receptor-9 (TLR9) is a cellular DNA-receptor and a member of the innate immune system which is widely expressed in breast cancers. Although synthetic TLR9-ligands stimulate breast cancer cell invasion in vitro, through down-regulation of tissue inhibitor of matrix metalloproteinase-3 (TIMP-3), the role of this protein in breast or other cancer pathophysiology is unclear. We discovered that low tumor TLR9 expression is associated with a significantly shortened breast cancer specific survival among patients with triple negative tumors, but not among those with ER+ tumors. We further discovered that hypoxia induces TLR9 expression in breast cancer cells in vitro and in vivo. Although the triple negative TLR9 siRNA MDA-MB-231 breast cancer cells (with lower TLR9 expression) were less invasive than control siRNA cells in normoxia, these cells became highly invasive in hypoxia. Hypoxia induces TLR9 expression in breast cancer cells in vitro and in vivo. Although the triple negative TLR9 siRNA MDA-MB-231 breast cancer cells (with lower TLR9 expression) were less invasive than control siRNA cells in normoxia, these cells became highly invasive in hypoxia. Although synthetic TLR9-ligands stimulate breast cancer cell invasion in vitro, through down-regulation of tissue inhibitor of matrix metalloproteinase-3 (TIMP-3), the role of this protein in breast invasion and pathways that cause EGFR overexpression. In addition, it may suggest that gefitinib therapy is useless in TNBC patients because we did not find any mutations in the tested exons of TNBC. EGFR overexpression may associate with a poor outcome of TNBC patients which suggest it could be a significant prognostic factor for TNBC patients. EGFR may play important role for molecular-targeting therapy of TNBC.
P2-11-11

IGFBP Ratio Confers Resistance to IGF Targeting and Correlates with Increased Invasiveness and Poor Outcome in Breast Tumors.
Becker MA, Hou X, Harrington SC, Carboni JM, Gottardis MM, Haluska P, Mayo Clinic, Rochester, MN; Bristol Myers Squibb Pharmaceutical Research Institute, Princeton, NJ

Purpose: To assess the role of insulin-like growth factor binding protein 5 (IGFBP-5) as a marker of relapse and survival in breast cancer tumors.

Experimental Design: Targeted regulation of IGFBP-5 was identified in MCF-7 cells resistant (MCF-7R4) to the IGF-1R/InsR inhibitor, BMS-536924 and examined by comparative microarray analysis, western and ELISA. Protein expression of IGFBP-5 was measured by immunohistochemistry in a cohort of 84 breast cancer patients to examine correlative associations with invasive tumor fraction and overall survival (OS). The expression ratio of IGFBP-5/IGFBP-4 (BPR) was determined in multiple breast tumor cohorts for univariate analysis.

Results: IGFBP-5 was markedly upregulated and highly localized to the membrane in MCF-7R4 resistant cells. When compared to pathologically normal reduction mammoplasty tissue, IGFBP-5 expression levels were upregulated in both invasive and histologically normal adjacent breast cancer tissue. In an independent cohort of breast cancer patients, IGFBP-5 protein levels correlated directly with invasion and OS. In univariate and multivariate modeling, metastasis-free survival, recurrence free survival (RFS) and OS were significantly associated with high IGFBP-5 expression. Prognostic power of IGFBP-5 was further increased with the addition of IGFBP-4 and tumors were ranked based upon IGFBP-5/IGFBP-4 expression ratio (BPR). Multiple breast cancer cohorts confirm that BPR (high vs. low) was a strong predictor of RFS and OS.

Conclusion: IGFBP-5 expression is a marker of poor outcome in breast cancer patients. An IGFBP-5/IGFBP-4 expression ratio may serve as a surrogate biomarker of IGF pathway activation and predict sensitivity to IGF-1R-targeted therapies.

P2-12-01

Immunohistochemical (IHC) BAG1 Expression Improves the Estimation of Residual Risk (RR) by IHC4 in Postmenopausal Patients Treated with Anastrozole or Tamoxifen: A TransATAC Study.
Dowsett M, Afentakis M, Pineda S, Salter J, Howell A, Buzdar A, Forbes JF, Cuzick J, Royal Marsden Hospital, London, United Kingdom; Wolfson Institute of Preventive Medicine, London, United Kingdom; Christie Hospital, Manchester, United Kingdom; MD Anderson Cancer Center, Houston; University of Newcastle, Newcastle, Australia

Aim: To determine whether the incorporation of BAG1 staining improves the estimate of RR after endocrine therapy in postmenopausal patients with ER+ve tumours treated with endocrine therapy.

Background: BAG1 encodes a protein (BCL2-associated athanogene 1) that binds to BCL2 and enhances its anti-apoptotic effects. BAG1 is included as a separate subgroup in the 21-gene OncotypeDX Recurrence Score (RS) that is used to assess RR after endocrine therapy in primary ER+ breast cancer. IHC4 is a 4-panel set of IHC markers (ER, PgR, HER2, Ki67) that was shown to provide as much prognostic accuracy as RS in the translational arm of the ATAC trial (TransATAC) of anastrozole versus tamoxifen alone or combined and subsequently independently validated (Cuzick et al, JCO, 2011, in press). Addition of extra markers such as BAG1 to IHC4 may improve the accuracy of the IHC4 and provide extra discriminatory power for oncologists.

Methods: Samples in triplicate TMAs from the TransATAC cohort were stained for BAG1 using the Genetex 3.10G3E2 antibody after validation using siRNA knockdown. Staining was scored separately as nuclear or cytoplasmic and categorized by intensity as 0, 1, 2 or 3. BAG1 IHC values were assessed for their correlation with BAG1 mRNA levels. The statistical analysis plan was pre-specified and tested possible additional information from BAG1 expression to the IHC4 in patients not treated with chemotherapy by change in the likelihood ratio chi-square (ΔLR-X2). Results were included only if there was also complete data for ER, PgR, Ki67 and HER2. Primary analysis was on the HER2-ve node-negative (N-neg) population; secondary analysis was on all N-neg patients. Follow-up was to 10 years and the primary end-point was time to distant recurrence (TTDR).

Results: Data on both nuclear and cytoplasmic BAG1 as well as the other 4 IHC parameters was available on 961 cases of which 855 were HER2-ve. There was a significant correlation between cytoplasmic and nuclear BAG1 (p=0.23, p=0.0001) but the nuclear staining correlated better with mRNA levels and was therefore considered further. Weak but significant correlations were also seen with ER, PgR and tumour grade. In the univariate analysis nuclear BAG1 was significantly associated with worse TTDR in HER2-ve and all N-neg cases (X2=7.91, p=0.005 and X2=10.63, p=0.001 respectively). Nuclear BAG1 also contributed significantly in multivariate analyses in the 2 populations firstly when added to the clinical model (X2=4.99, p=0.02 and X2=5.93, p=0.015 respectively) and secondly when subtracted from clinical plus the IHC4 parameters (X2=5.55, p=0.02 and X2=4.50, p=0.03 respectively).

Conclusions: Nuclear BAG1 expression has significant value for estimating RR that is independent of standard clinical and IHC parameters and it improves the prediction of TTDR in the TransATAC population beyond that with the validated IHC4 score. Unlike IHC4 markers, BAG1 is not commonly measured in pathology work-up of breast cancers. The clinical utility of its addition to IHC4 will be tested by measuring its discrimination of high and low risk patients in clinical practice.
trial. Pts were more likely to be older and postmenopausal (p=0.0001 for both). There was no significant association between BMI and ER/PR status (p=0.07) or histologic tumor grade (p=0.33). Obese pts were found to have significantly larger tumors ≥ 2 cm (p=0.002) and more positive lymph nodes (p=0.02). There was no significant difference in DFS within each intrinsic arm (A, B and C) between the obese and non-obese pts at 3, 5 or 7 yrs of follow up. However, pts in the non-obese group had significantly improved DFS in arm B and C compared to arm A (p=0.001 and p=0.0001 respectively). Also obese pts in arm C had significantly improved DFS compared to obese pts in arm A (p=0.008). There was a trend of improved DFS in the obese group in arm B compared to arm A, but this was not statistically significant (p=0.09). Pts in the normal weight and overweight groups did significantly better in arm B (p=0.02 for both) and arm C (p=0.01 and p=0.002 respectively) compared to arm A.

Conclusions: This analysis of data from the N9831 study confirms that obese pts with early stage HER2+ tumors have worse clinical outcome than pts with BMI < 30%. Adjuvant trastuzumab improved clinical outcome regardless of BMI. This study supports weight loss intervention for obese women with early stage HER2+ BC.

P2-12-03
A Prospective Study of the Prognostic Implications of Being a BRCA1 Carrier for Young Onset Breast Cancer Patients.
Eccles DM, Dent L, Gerty SM, Altman D, Copson ER, Simmonds PD, Duncan L, Ward D. University of Southampton, Southampton, Hampshire, United Kingdom; Oxford University, Oxford, United Kingdom; Southampton University Hospitals NHS Trust, Southampton, Hampshire, United Kingdom; Salisbury District Hospital, Salisbury, Wiltshire, United Kingdom

Background: BRCA1 gene carriers frequently develop triple negative breast cancer (TNBC) at young ages. Retrospective studies have reached conflicting conclusions about the prognostic implications of breast cancer diagnosed on a background of a constitutional BRCA1 gene mutation. Many studies have the disadvantages of retrospective design and small numbers or both. The Prospective study of Outcomes in Sporadic versus Hereditary breast cancer (POSH) recruited 3024 patients diagnosed before 41 years of age and treated for breast cancer in the UK between 2000 and 2007 (protocol published: Eccles et al, BMC Cancer 2008).

Aim: To investigate the effect of family history and BRCA1 gene mutation status on breast cancer survival in women aged younger than 41 years at diagnosis.

Methods: Cases in which the primary tumour pathology report was not available (2.8% of total) were excluded from this analysis. A completed family history questionnaire was available for 2907 cases (99% of total). The primary end point for this analysis was the development of distant metastases from breast cancer. All patients were younger than the starting age for screening in the UK except for known gene carriers of which there were only 8 in total, the remainder of identified gene carriers were tested after cancer diagnosis or as part of this study. Over 90% of patients received adjuvant or neoadjuvant chemotherapy with over 90% of these receiving an anthracycline based regimen. Kaplan Meier survival analysis and log rank test were used to assess survival differences.

Results: 2937 patients were included in the analysis. Mean and median age at diagnosis was 36 years and the cohort was followed prospectively for a mean and median follow up time of 50 months. Overall distant disease free survival in the whole cohort was only 75% at 5 years. The effect of family history on presentation was explored. Patients who reported a first or second degree relative with breast cancer presented with smaller tumours (20mm compared to 22.5mm, p= 0.006) but no difference in axillary nodal status (p=0.2605). Survival was however significantly better comparing the 499 patients who reported a family history of breast cancer compared with the rest of the cohort (p=0.0164). 539 patients had been tested for mutations in the BRCA1/2 genes and 176 high risk gene BRCA1/BRCA2 carriers were identified. We compared 118 patients with a known BRCA1 gene mutation (almost all diagnosed after the cancer) to the 465 patients with confirmed TNBC (p=0.0118). Survival was significantly better in the BRCA1 carriers (p=0.0118). After excluding 29 gene carriers whose primary tumour showed any of ER, HER2 or PR as positive, the same improved survival trend was seen although significant at the 10% level (p=0.07).

Conclusion: This prospective study indicates that BRCA1 gene carriers clearly do not have a worse prognosis when compared to triple negative breast cancers or young onset breast cancer overall and these early follow up data indicate an improved outlook in gene carriers.

P2-12-04
RACGAP1 mRNA Assay Outperforms Ki-67 as a Proliferation Marker in the FinHer Study Cohort.
Wirtz RM, Leinonen M, Bono P, Isola J, Kellokumpu-Lehtinen P-L, Kataja V, Turpeenniemi-Hujanen T, Jyrkkö S, Huang W, Eitd S, Joensuu H. STRATIFYER Molecular Pathology GmbH, Cologne, Germany; Pharma, Finland; Helsinki University Central Hospital and University of Helsinki, Finland; University of Tampere and Tampere University Hospital, Finland; Tampere University Hospital, Finland; Kuopio University Hospital, Finland; Oulu University Hospital, Finland; Turku University Hospital, Finland; Monogram Biosciences, Inc.; Institute of Pathology at the St-Elisabeth-Hospital, Germany

Background: Molecular subtyping of breast cancer has become an integral part of standard evaluation of breast cancer patients. Their assessment requires combining data from analyses on ER, PR, HER2 and cell proliferation markers. However, their immunohistochemical (IHC) testing carries an up to 20% risk of erroneous results. Similarly, assessment of cell proliferation by Ki-67 staining is hampered by lack of standardization of laboratory methods and agreement on cut-offs. Here we tested the prognostic value of objective quantitation of ESR1, PgR, HER2 and the proliferation markers RACGAP1 using RT-qPCR and compared the results with local and central IHC assessments.

Methods: RNA was extracted from FFPE tumor tissue of 917 patients who participated in the FinHer trial. ESR1, PgR, HER2 and the proliferation marker RACGAP1 using RT-qPCR and compared the results with local and central IHC assessments.

Results: HER2 mRNA expression increased in parallel with HER protein expression. The WHO BMI classification parameters of < 25%, 25-29% and ≥ 30% respectively were grouped into non-obese (BMI< 30) and obese (≥ 30) cohorts. DFS was estimated by the Kaplan-Meier method. Comparisons between arms A (chemotherapy alone), B (chemotherapy plus sequential trastuzumab) and C (chemotherapy plus concurrent trastuzumab) were performed using the Cox proportional hazards model, stratified by BMI.

Conclusions: This prospective study indicates that BRCA1 gene carriers clearly do not have a worse prognosis when compared to triple negative breast cancers or young onset breast cancer overall and these early follow up data indicate an improved outlook in gene carriers.
central IHC and CISH was good, while local IHC testing suffered higher false positive rates. RACGP1 mRNA expression was the greater the higher the histological grade. ESR1 and PgR mRNA correlated negatively with the histological grade (r=-0.38 and r=-0.33; p<0.0001), whereas HER2 and RACGP1 mRNA were correlated positively (r=0.10 and r=-0.49; p=0.002 and p=0.0001, respectively). RACGP1 mRNA was negatively associated with ESR1 and PgR mRNA (r=0.17 and r=-0.26, respectively; p=0.0001 for each). Molecular subtypes determined by RT-qPCR using predefined cut-off values were highly prognostic for overall survival (OS) (p<0.001). The 5-year OS rate for patients with luminal cancer was 94% and 86% for HER2-enriched cancer and 84% for triple-negative cancer. In the subset of luminal tumors, high expression of RACGP1 identified a population of patients who were at a high risk of death (5-year OS 82% versus 95%; p=0.0001). In a multivariate analysis RACGP1 mRNA expression, nodal status and chemotherapy type were independent prognostic factors, whereas IHC of ER, PgR, Ki-67 and histological grade were not significant.

Conclusion: Molecular subtyping of breast cancer by RT-qPCR using RNA isolated from FFPE tissue proved successfully in this large patient cohort. RACGP1 mRNA expression distinguished high and low risk luminal breast cancers. In a multivariate analysis mRNA-based molecular markers outperformed the immunohistochemical markers ER, PgR and Ki-67. Of note, quantitative assessment of the proliferation marker RACGP1 was superior to semi-quantitative assessment of Ki-67 from routine FFPE tissues using IHC. We conclude that quantitative assessment of ESR1, PgR, HER2 and RACGP1 mRNA by RT-qPCR is a robust and reproducible method to assess these key tumor biological factors from archival FFPE tumor tissue. RACGP1 is a novel cell proliferation marker in breast cancer that warrants further validation.

P2-12-05
Correlation between Quantitative HER2 Protein Expression and Risk of Brain Metastases in HER2-Positive Advanced Breast Cancer Patients Receiving Trastuzumab-Containing Therapy.

Duchnowska R, Biernat W, Szostakiewicz B, Sperinde J, Piette F, Hauck M, Paquet A, Lie Y, Czartoryska-Artukowicz B, Wysocki P, Jankowski T, Radecka B, Foszczyńska-Kłoda M, Litwinik M, Debska S, Weidler J, Huang W, Buyse M, Bates M, Jassem J. Military Institute of Medicine, Warsaw, Poland; Medical University of Gdansk, Gdansk, Poland; Monogram Biosciences, South San Francisco, CA; International Drug Development Institute, Louvain-la-Neuve, Belgium; Bialystok Oncology Center, Bialystok, Poland; Great Polish Cancer Center, Poznan, Poland; Lublin Oncology Center, Lublin, Poland; Opole Oncology Center, Opole, Poland; West Pomeranian Oncology Center, Szczecin, Poland; Poznan University of Medical Sciences, Poznan, Poland; Regional Cancer Center, Lodz, Poland; Cepheid, Sunnyvale, CA

Background. Patients with HER2-positive breast cancer are at particularly high risk for brain metastases; however, the biological basis is not fully understood. Within HER2-positive breast cancer tumors, it is possible to resolve a ~1.5-log range of HER2 protein expression using a novel quantitative HER2 assay (HERmark®). We investigated the correlation between quantitative HER2 protein expression in primary breast cancers and the time to brain metastases (TTBM) in HER2-positive advanced breast cancer patients treated with trastuzumab.

Methods. The study group included 142 consecutive patients who were administered trastuzumab-based therapy for HER2-positive metastatic breast cancer, defined as 3+ categorical staining by immunohistochemistry (IHC). HER-2/neu gene copy number was subsequently quantified as HER2/CEP17 ratio by central laboratory fluorescence in situ hybridization (FISH). HER2 protein was quantified as total HER2 protein expression (H2T) by the HERmark assay in formalin-fixed, paraffin-embedded primary tumor samples. HER2 variables were correlated with clinical features and TTBM measured from the initiation of trastuzumab-containing therapy. Results. H2T level (continuous variable) was correlated with shorter TTBM (HR=2.3; p=0.013), whereas HER2 gene amplification by FISH (p=0.28) and continuous HER2/CEP17 ratio (p=0.25) had no significant prognostic impact. The correlation between continuous H2T level and TTBM was confirmed in a multivariate analysis (HR=3.2; p=0.021). Controlling for the competing risk of death from causes other than brain metastases, continuous H2T remained a strong correlate of TTBM (HR=2.7; p=0.0009). In the subset of patients that was centrally-determined HER2 positive by FISH (117 patients), above-median H2T level was significantly associated with shorter TTBM (HR=2.4; p=0.005), whereas this was not true for median FISH/CEP17 ratio (p=0.4). In a multivariate analysis of this subset, continuous H2T (p=0.021) and a time dependent covariate capturing time to non-brain metastases (p=0.0044) were prognostic for TTBM, whereas FISH/CEP17, ER, PgR and grade were not.

Conclusion. These data reveal a strong relationship between quantitative HER2 protein expression levels and the risk of brain relapse in HER2-positive advanced breast cancer patients. Consequently, quantitative assessment of HER2 protein expression may inform and facilitate refinements in therapeutic treatment strategies for selected subpopulations of patients in this group.

P2-12-06
Nomogram To Predict Subsequent Bone Metastasis in Patients with Non Metastatic Breast Carcinomas.

Lousquy R, Delpech Y, Rouzier R, Glisovic J, Hsu L, Barranger E, Puzsztai L, Uzan S, Hori-bagyi GN, Costant C, Ibrahim NK, Lariboisiere Hospital, AP-HP, Paris, France, Metropolitan; Tenon Hospital, AP-HP, Paris, France, Metropolitan; The University of Texas M.D. Anderson Cancer Center, Houston, TX; Georges Francois Leclere Cancer Center, Dijon, France, Metropolitan

Background: Bone metastasis (BM) is one of the most common sites of distant metastasis for breast cancer. We hypothesized that its occurrence can be predicted if a clinical nomogram can be developed, thus allowing for selection of patients at high risk for BM.

Patients and Methods: Medical records of patients with non metastatic breast cancer were prospectively collected for the period between January 1997 and February 2007 at the M.D. Anderson Cancer Center (Texas, USA). A multivariate logistic regression analysis of selected prognostic features was done. Based on Cox proportional hazards regression model, a nomogram to predict BM was constructed and validated in an independent cohort of 579 patients with non metastatic breast cancer at time of diagnosis treated at the Tenon Hospital (Paris, France) between January 2003 and December 2005.

Results: Of 4175 patients with non metastatic breast cancer, 314 developed subsequent BM. Age, T stage, lymph node status, lymphovascular space invasion, breast cancer molecular subtype, adjuvant hormonotherapy were significantly and independently associated with subsequent BM. The nomogram had a concordance index of 0.69 (95% CI, 0.68 to 0.70) in the training set. The validation set showed a good discrimination with a concordance index of 0.65 (95% CI, 0.57 to 0.72). At 3, 5 and 7 years, the nomogram was well calibrated.
Conclusion: We have developed a robust tool that is able to predict subsequent BM in patients with non metastatic breast cancer. Selection of an enriched patient population at high risk for BM will allow to practice individualized therapeutic strategies, an adapted medical supervision and will facilitate the design of trials aiming at its prevention with the use of biphosphonate treatment.

P2-12-07
Pooled Analysis of Outcomes of T1a/bN0, HER2-Amplified Breast Cancer.
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Background: Breast tumors with HER2 amplification have a worse prognosis than those with normal HER2 expression. This finding is independent of tumor size and other classical prognostic factors used in the adjuvant setting. The prognosis of node negative, sub-centimeter (T1a/bN0) tumors is usually excellent. However, little is known about the prognostic impact of HER2-amplification in this group. This study therefore, aimed to evaluate the relative and absolute prognostic impact of HER2-amplification in T1a/bN0 tumors.

Materials and Methods: Published data from studies assessing the outcomes of patients with HER2-amplified, node negative, T1a/bN0 tumors were included in a pooled analysis. Odds ratios (OR), 95% confidence intervals (CI) and absolute risks were computed for recurrence and distant recurrence at 5 years. Pooled hazard ratios (HR) for disease-free survival (DFS), were also assessed.

Results: A total of 3 case-control studies were included in the analysis and comprised 485 patients with HER2-amplified breast cancer (57.3% were also hormone receptor positive) and 1096 patients with HER2-normal disease (82.2% were hormone receptor positive). Among the HER2-amplified group, 4.1% received trastuzumab and 18.6% received chemotherapy. In the HER2-normal group, 4.3% received chemotherapy. Estimated median follow-up was 5.7 years. HER2-amplification was associated worse DFS (HR = 2.60, 95% CI 1.53-4.41, p<.001) and increased odds for recurrence at 5 years (OR =3.79, 95% CI 2.35-6.10, p<.001). There was a non-significant trend towards increased odds of distant recurrence at 5 years (OR = 2.51, 95% CI 0.82-7.67, p=.11). Compared with HER2-normal cancers, those with HER2-amplification showed lower absolute probability of recurrence-free survival at 5 years (90.1% vs. 94.6%, p<.001) and distant recurrence-free survival at 5 years (95.1% vs. 97.6%, p<.001). Among HER2-amplified cancers, tumor size 0.6-1.0cm (T1b) was associated with a trend for higher odds of recurrence at 5 years compared with those 0.5cm or smaller (T1a, pooled OR = 1.58, 95% CI 0.96-2.60, p=.07).

Conclusions: HER2-amplification is associated with worse outcome in T1a/bN0 tumors. However, recurrence-free and distant recurrence-free survival at 5 years is excellent in this group, particularly in those with T1a tumors. These data question the role of adjuvant chemotherapy and trastuzumab in these patients unless associated with other high risk factors. A differentially lower risk for distant recurrence suggests the possible role for more aggressive local therapy such as surgery and/or radiation therapy.

P2-12-08
Bcl-2 as a Prognostic Marker in Breast Cancer Patients Receiving Endocrine Therapy.
Larsen MS, Bjerre KD, Løkkhuseth A-V; Giobbie-Hurder A, Ejertsen B, Lyksesfeldt AE, Rasmussen BB, Herlev Hospital, Herlev, Denmark; Rigshospitalet, Copenhagen, Denmark; Dana-Farber Cancer Institute, Boston, MA; Danish Cancer Society, Inst of Cancer Biology, Copenhagen, Denmark

Background: In breast cancer patients with estrogen receptor (ER) positive tumors, endocrine treatment reduces recurrence. However, 30% of patients eventually relapse in spite of therapy. Additional markers to identify this group of therapy resistant, prognostically unfavourable patients are therefore needed. The expression of Bcl-2 has been shown to be a prognostic factor in breast cancer patients regardless of ER-status, but assessment of Bcl-2 by immunohistochemistry (IHC) needs to be standardized since different scoring systems and different cut-points have been used. In a pilot study comprising 258 ER positive tumors from tamoxifen-treated patients, a simple scoring system enabled us to find suitable cut-points, selecting patient-groups with poor prognosis. The aim of the current study was to validate these results in a similar population from a large randomised study.

Material and methods: Bcl-2 was assessed in 1082 (78%) of the 1396 postmenopausal Danish patients who in the BIG 1-98 trial were randomised to 5 years of tamoxifen (N=325), letrozole (N=335) or a sequence of these agents (N=422). Tissue microarrays were made from formalin fixed paraffin embedded primary tumors. An IHC score for expression of cytoplasmic Bcl-2 (clone 124, Dako) was performed according to both the number of positive cells (0%=0, 1-10%=1, 11-50%=2, 51-100%=3) and the intensity of the staining (weak, moderate, strong) as in the pilot study. The associations between Bcl-2 status and clinicopathological variables were assessed. The prognostic value of Bcl-2 according to cut-points determined in the pilot study was analysed by Cox proportional hazards models. The primary endpoint was disease free survival (DFS).

Results: Patients were divided into three subgroups according to predefined cut-points: Bcl-2 low (score 0 or 1 (n= 24)), Bcl-2 intermediate (score 2 or score 3/weak or moderate (n= 216)) and Bcl-2 high (score 3/strong (n= 842)). Bcl-2 expression was significantly associated with ER (P=0.0003) and progesterone receptor (PgR) (P<0.0001), and negatively associated with Ki-67 (P=0.0021) and tumor grade (P=0.005). In univariate analysis patients in the Bcl-2 low group had a bad prognosis compared to the Bcl-2 high group (HR=2.3; 95% CI: 1.2 to 4.1). In the multivariable analysis including ER, PgR, Ki-67, positive lymph nodes, age, tumor size and histological type and grading, association between Bcl-2 and DFS was not statistically significant (P=0.35).

Discussion: We found Bcl-2 to be a prognostic factor in the univariate analysis (P=0.024) but not to be an independent predictor of poor survival of ER positive patients treated with tamoxifen and/or letrozole. In this population a low or absent expression of Bcl-2 was uncommon (2.2 %), as expected since Bcl-2 is an ER regulated protein. The results of the pilot study could not be confirmed in this validation study. However, the strong positive relation to other prognostic factors like tumor grade and high proliferative index indicates that Bcl-2 could contribute to factors characterising a group of tumors with a bad prognosis.
P2-12-09
Prediction of Residual Risk of Recurrence for 5 Years of Follow-Up by Clinicopathologic Variables and 4 IHC Markers: A TransATAC Study.
Sestak I, Cuzick J, Dowsett M, Salter J, Quinn E, Zabaglo L, Howell A, Buzdar A, Forbes J, Queen Mary University of London, London, United Kingdom; Royal Marsden Hospital and Breakthrough Breast Cancer Centre, London, United Kingdom; Christie Hospital, Manchester; United Kingdom; University of Texas, Texas; University of Newcastle, Newcastle, NSW, Australia

Background: Adjuvant endocrine therapy beyond 5 years is known to be of benefit to some ER+ patients but it is unclear which patients have sufficient residual risk (RR) to merit this. We have previously shown that 4 immunohistochemical markers (ER, PgR, Ki67, HER-2), both alone and combined into the IHC4 score (Cuzick et al, JCO 2011) are significantly correlated with time to recurrence (TTR) in the overall follow up of a cohort of 1125 patients from the monotherapy arms of the Arimidex, Tamoxifen, Alone or in combination (ATAC) trial. We have now assessed the relationship of each of these parameters and common clinical variables (nodal status, grade, tumour size, age and treatment option) for predicting outcome beyond 5 years.

Material and Methods: We determined the univariate and multivariate prognostic value of clinical variables and the 4 IHC variables separately and as the IHC4 score for TTR separately in years 0-5 and 5-10 of follow up for all patients, separately for anastrozole and tamoxifen and only in the node-negative patients. Results: Results in years 5-10 are summarized in the Table. Nodal status, tumour size and grade were at least as strong in years 5-10 as in years 0-5. Ki67 and the overall IHC4 score were the only significant IHC biomarkers related to TTR univariately in this period, but both lost significance in a multivariate model including clinical variables. There were no significant interactions with treatment. Similar results were seen for the node-negative population. Conclusions: None of the IHC4 markers provided significant additional prognostic information in the 5-10 year period, but nodal status, tumour size and grade continued to be strong prognostic factors.

Table: Univariate and multivariate analyses of immunohistochemical markers and clinical variables in the 5-10 years follow-up period.

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<td>Tumour size</td>
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<tr>
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<td>0.93 (0.73-1.18)</td>
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<td>Grade</td>
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<tr>
<td>(well vs. mod./poor)</td>
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<td>0.73 (0.61-0.89)</td>
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<tr>
<td>Clinical Score</td>
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<tr>
<td>(H-Score/50)</td>
<td>0.92 (0.61-1.40)</td>
<td>0.31 (0.15-0.61)</td>
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<td>ER (5%10)</td>
<td>0.89 (0.62-1.26)</td>
<td>0.74 (0.50-1.09)</td>
</tr>
<tr>
<td>HER2 (pos vs neg)</td>
<td>0.99 (0.59-1.62)</td>
<td>0.74 (0.46-1.17)</td>
</tr>
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<td>IHC4 Score</td>
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<td></td>
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<tr>
<td>(Ki67=10%+P50/67%+ER)</td>
<td>1.29 (0.99-1.70)</td>
<td>0.96 (0.61-1.47)</td>
</tr>
<tr>
<td>(P&lt;0.001)</td>
<td></td>
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</tr>
</tbody>
</table>

* For addition to model containing all other factors (except clinical score and IHC4 score which only is for addition to the other score)

P2-12-10
Low TCR Diversity (Divpenia) Is a Prognosis Factor of Overall Survival in Metastatic Breast Cancer.

Background: We already showed that lymphopenia (<1000 lymphocytes/µL) or CD4+ T cell lymphopenia (<450/µL) detected before initiation of chemotherapy are prognostic factors for toxicity and death for metastatic breast cancer (MBC) patients. The goal of the present study was to identify the characteristics of the T cells in these lymphopenic patients. TCR diversity was investigated and tested as a prognostic factor for overall survival (OS).

Patients and methods: The ImmunTraCkeR® assay (ImmunID, Grenoble, France), which analyzes through semi quantitative multiplex-PCR the V-D-J combinatorial diversity of TCR-beta chain (TRB), was used to investigate diversity of T cell repertoire on cryopreserved blood samples from a retrospective cohort of MBC patients before chemotherapy administration (n=66). Univariate and multivariate analyses were performed. We then validated our score on a prospective cohort (n=67) using the same eligibility criteria (MBC patients before first line chemotherapy administration).

Results: Using a 33% cutoff for divpenia in our retrospective cohort (T cell diversity below 33%) (average diversity for healthy people is 70%), divpenia was associated with a median OS of 10 months vs 22 months for patients with diversity >33% (logrank p value=0.04). The NDLL score (Numeration Diversity Lymphocytes representation) that combines lymphocyte numeration with TRB diversity, demonstrated that lympho-divpenia (T cell diversity below 33% and lymphopenia below 1Giga/L) was associated with a poor OS compared to patients with either lymphocyte <1000/µL & diversity >33% or lymphocyte >1000/µL & diversity <3% or both lymphocyte >1000/µL & diversity >33% (p=0.015).

In multivariate analysis, including performance status (PS), hemoglobin level, polynuclear neutrophil count (PNN), age, and liver metastasis, NDLL score was identified as an independent prognostic factor for OS. In our prospective validation cohort, NDLL score was also identified as a prognostic factor for OS (p=0.007), as well as lymphopenia (<1000/µL) (p=0.0003), CD4+ lymphopenia (<450/µL) (p=0.04), menopausal status (p=0.02), hormonal receptor status (estrogen receptor p=0.02; progesterone receptor p=0.002) and lung metastasis (p=0.009).

In multivariate analysis, hemoglobin level was the only independent prognostic factor in this cohort.

Conclusion: We showed that Divpenia and NDLL score are prognostic factors for OS in MBC patients. In order to confirm these results, a prospective clinical trial is ongoing on a larger cohort of MBC and lung cancer patients.

P2-12-11
Clinical Relevance of a IL-8/B-Cell Gene Signature Identified from Triple Negative Breast Cancer (TNBC) in Intrinsic Breast Cancer Subtypes.
Rody A, Holtrich U, Ruckhaeberle E, Radosa J, Juhász-Boess I, Solomayer EF, Kaufmann M, Karrn T, Starland University, Hamburg, Germany; Goethe-University, Frankfurt, Germany

Background: As presented recently (SABCS 2010, #S5-5) a ratio of high B-cell and low IL-8 metagenes using gene expression analysis identified 32
% of triple negative breast cancers with good prognosis and was the only significant predictor in multivariate analysis including routine clinicopathological variables. However, the clinical relevance of this signature within the intrinsic breast cancer subtypes still remains unclear and is analyzed here.

**Methods**

Affymetrix gene expression data from n=2417 breast cancer patients have been assembled. We performed an unsupervised analysis to define metagenes that distinguish molecular subsets within TNBC (SABCS 2010, #S5-5). A high expression of B-cell metagenes was associated with good and high expression of IL-8-related metagenes were associated with poor prognosis. To identify intrinsic subtypes we used the method previously described by Hu et al. (2006) and the prognostic value within those subtypes was assessed by analyzing the event free survival of patients as function of high and low B-cell/IL-8 metagene ratio.

**Results**

Comparing ER positive with ER negative patients the B-cell/IL-8 ratio showed only in ER negative breast cancer a significant prognostic value (log rank p-value <0.0001). Within the entire cohort 37.8 % of patients could be assigned to luminal A, 35.2 % to luminal B, 7.4 % to erbB2 and 19.6 % to basal-like subtypes. Event free survival of patients with good or poor B-cell/IL-8 ratio showed only in basal-like breast cancer patients a statistical significant difference (p<0.0001). However, we could not observe any difference in prognosis in luminal A and B, as well as erbB2 tumors. No difference in the expression of the proliferation metagene was observed when samples of the intrinsic subtypes were stratified according to the prognostic predictor based on high expression of the B-Cell metagene and low expression of the IL-8 metagene.

**Conclusion**

The B-cell/IL-8 ratio is highly prognostic in basal-like/ TNBC and shows no association with proliferation status.

**P2-12-12**

**Prognostic Utility of Breast Cancer Index for Late Relapse in Patients with Early Stage Breast Cancer.**

**Schnabel CA, Zhang Y, Kesty NC, Erlander MG. bioTheranostics, Inc, San Diego, CA**

**Background:** Residual risk of relapse remains a substantial concern for breast cancer patients as greater than half of recurrences occur beyond the initial 5y of tamoxifen therapy. First generation multi-gene signatures provide further prognostic information to standard clinical and pathological factors, however, their utility is strongest for predicting early relapse (≤5y post-diagnosis), and they have limited prognostic value for late metastatic risk. Breast Cancer Index (BCI), a continuous risk index based on the combination of HOXB13/IL17BR (H1) and the molecular grade index (MGI), estimates the individual risk of recurrence in ER+, LN- breast cancer patients. In this study, the prognostic performance of BCI for predicting early versus late relapse (≥5y vs >5y post-diagnosis) was examined.

**Methods:** Gene expression profiling was performed on RNA extracted from FFPE tumor samples from untreated, postmenopausal, ER+ early stage breast cancer patients in the randomized Stockholm Trial. RT-PCR assay, pre-defined BCI score, H1 and MGI cut-points, and risk group categorization were done as previously described (Jerevall et al., Br J Cancer 2011). Association of gene expression data with the clinical endpoint of time to distant metastasis was assessed by Kaplan-Meier analysis using the log rank test; time-varying coefficient Cox proportional models were used to estimate the time-dependent hazard ratios (HRs).

**Results:** Analyses included 274 ER+, LN- patients (51% PR+, 87% HER2+, 63% grade 2, 17.7 y median follow-up) who did not receive adjuvant tamoxifen treatment. BCI was significantly associated with 10-year distant metastasis-free survival, with probabilities of 91% (86-96%), 82% (74-91%), and 65% (52-80%) for the low, intermediate, and high-risk BCI groups, respectively (HR high versus low-risk group = 4.31; 95%CI: 2.23-8.33; P=0.00001). Stratification for the first 5-y post-diagnosis using time-varying coefficient Cox models showed both MGI and BCI were significantly prognostic with HRs of 6.13 (95% CI: 2.11-17.8; P=0.0009) and 5.77 (95% CI: 2.16-15.39; P=0.0005). For prediction of late relapse in the subset of patients that remained distant metastasis-free for at least 5-y (N=221), MGI decreased in prognostic utility (HR 1.65, 0.76-3.56; P=0.2), consistent with other proliferation-based gene signatures. In comparison, both H1 and BCI were significantly associated with risk of late relapse [HRs 2.89 (1.31-6.36; P=0.009); 3.31 (1.3-8.39; P=0.012)].

**Conclusions:** This post-hoc analysis of a randomized clinical trial cohort demonstrates the prognostic utility of BCI to predict disease outcome for both early and late risk of relapse in untreated patients with early stage breast cancer. Given the significant need for predictors of late risk, the stability of BCI prognostic performance may have important implications for the type and duration of treatment for hormone-responsive breast cancer.

**P2-12-13**

**Topoisomerase 2 alpha (TOP2A) RNA Expression Provides Prognostic Information in Hormone Receptor Positive Breast Cancer That Is Complementary to a Simulated Algorithm for Recurrence Score.**

**Sparano JA, Goldstein LJ, Davidson NE, Sledge, Jr GW, Gray R. Albert Einstein College of Medicine, Montefiore Medical Center, Bronx, NY; Fox Chase Cancer Center, Philadelphia, PA; University of Pittsburgh, Pittsburgh, PA; Indiana University, Indianapolis, IN; Eastern Cooperative Oncology Group, Brookline, MA**

**Background:** We have previously reported that TOP2A RNA expression was prognostic in patients with operable estrogen receptor (ER) positive breast cancer treated with anthracycline containing adjuvant chemotherapy plus endocrine therapy in trial E2197, and provided complementary prognostic information in those who had a mid-range Recurrence Score (RS) (Sparano et al. Clin Cancer Res 2009; 15; 7693). The purpose of this analysis was to provide additional evidence regarding this observation.

**Methods:** We evaluated TOP2A RNA expression in 4 independent data sets and stratified patients with early stage breast cancer into low (≤ Median RS), intermediate (Median RS to 18 RS), and high RS (>18 RS) groups. Patients were also stratified by ER status, stage, grade, menopausal status, and type of adjuvant therapy (chemotherapy, tamoxifen, both). The impact of TOP2A expression on relapse-free survival was assessed using Cox proportional hazards models.

**Results:** TOP2A expression was associated with improved relapse-free survival in patients with ER positive breast cancer treated with adjuvant chemotherapy plus endocrine therapy. TOP2A expression was strongly associated with improved recurrence-free survival in patients with low RS (HR 0.3; 95% CI 0.2-0.5) and modestly improved recurrence-free survival in patients with intermediate RS (HR 0.6; 95% CI 0.4-0.9). TOP2A expression was not associated with improved recurrence-free survival in patients with high RS (HR 0.8; 95% CI 0.5-1.3). TOP2A expression was associated with improved relapse-free survival in patients with low RS, but not in patients with intermediate RS or high RS.
TOP2A expression was significantly associated with recurrence in all 4 datasets when evaluated individually (HR 1.56, p<0.001 in pooled analysis), and 2 of 4 datasets individually when adjusted for SimRS (HR 1.26, p=0.003 in pooled analysis). SimRS was significantly prognostic when adjusted for TOP2A individually in 3 of 4 datasets (HR 1.44, p<0.0001 in pooled analysis). For those with an intermediate SimRS, five year RFS rates were significantly higher in 2 datasets individually for high vs. low TOP2A expression (HR 1.82, p=0.007 in pooled analysis).

Conclusions: This analysis provides additional evidence that TOP2A expression provides prognostic information in patients with ER-positive, HER2-negative disease, a population known to have low incidence of TOP2A gene alterations. These findings also suggest that TOP2A expression provides information that is complementary to RS, and may be useful for identifying high risk subjects who have a mid-range RS. These findings require prospective validation in other prospective or prospective-retrospective trials using the actual rather than simulated RS.

P2-12-14
Prognostic Value of HER2 Positivity and Negative Hormonal Status in Patients with Small Tumor (<1cm) and Node-Negative Breast Cancer.
Meatini I, Livi L, Saieva C, Agresti B, Scotti V, Nori J, Sanchez LJ, Vezzosi V, Bianchi S, Cataliotti L, Bitt G. Florence University, Florence, Italy; Cancer Prevention and Research Institute, Florence, Italy

Introduction. Human epidermal growth factor receptor 2 (HER2) amplification has become the prototype biomarker to develop tailored biological treatment. Many studies suggested that HER2 positivity is an independent predictor of disease recurrence and breast cancer mortality. Trastuzumab has been introduced into clinical practice for high-risk HER2-positive patients who have completed the standard adjuvant treatment. However, small (<1cm), node-negative tumors remain a subgroup of HER2-positive patients who are currently ineligible for trastuzumab treatment, as clinically they have been deemed to have no requirement for standard adjuvant chemotherapy.

The aim of our analysis was to evaluate the prognostic factors of local and distant recurrence in patients diagnosed with T1a-b, node-negative, HER-2 positive breast cancer.

Materials and methods. A total of 704 patients were diagnosed at Florence University between November 1999 and December 2008 with node-negative, invasive BC that were 1 cm or smaller. Patients with ductal carcinoma in situ, with recurrent BC at presentation and patients that received adjuvant chemotherapy were excluded from analysis.

Results. Mean follow-up was 4.9 years (0.5 – 10.8 years); we recorded a total of 19 recurrences, including 10 distant recurrences. Mean time to local relapse occurrence was 3.8 years (0.4 – 7.3 years); mean time to distant metastases diagnosis was 4.4 years (1.4 – 7.6 years). Among all patients, the recurrence-free survival (RFS) was 93.7%.

The only parameter that emerged as significant predictor of events is the age (p=0.02).

The distant recurrence-free survival (DRFS) rate was 96.5%. Patients who had HER2-positive BC had worse DRFS than patients who had HER2-negative BC (92.0% vs 96.9%; p=0.045). In addition to HER2, also HR status was significantly associated with DRFS (p=0.026).

Patients who had HR negative status had worse DRFS than patients who had HR positive status (91.4% vs 97.4%; p=0.045).

Conclusions. Patients with T1a-b, node-negative, breast cancer have a low risk of distant and local recurrence. Women with HER2-positive and negative hormonal status have a significant risk of distant recurrence and should be considered for anti-HER2 adjuvant therapy.

P2-12-15
Dorairaj JJ, Wall D, Newell J, Blamey RW, Sweeney KJ, Ball G, Kerin MJ. National University of Ireland, Galway, Ireland; Breast Institute, Nottingham City Hospital, Nottingham, United Kingdom; Nottingham Trent University, Nottingham, United Kingdom

Introduction: Axillary lymph node status is an important predictor of overall survival (OS), hence its inclusion in clinical prognostic tools. The Nottingham Prognostic Index (NPI) which incorporates Lymph Node (LN) stage, tumor size and grade generates a score which predicts a percentage 10-year survival. Despite its status as a benchmark model for breast cancer prognosis, newer prognostic factors do exist. Lymph Node Ratio (LNR) is a superior prognostic indicator compared to absolute positive lymph node number, warranting re-evaluation of breast cancer prognostication. The aims of this study were threefold: identify the strength of LNR as a prognostic indicator compared to LN stage and NPI; establish a new prognostic index (Galway Index of Survival [GAINS]), taking into account the effect of LNR and breast cancer subtype on traditional clinicopathological features in breast cancer prognostication; and evaluate the prognostic efficacy of the new index compared to NPI.

Methods: Two cohorts were used: Galway Cohort—a prospectively compiled cohort of 1668 cases with histologically proven Stage 1, 2 and 3 primary operable breast cancer treated between 1990-2010 in a single institute; and ONCOPOOL—a retrospectively compiled database of 16944 cases treated across 12 European breast cancer units between 1990-1999. A Cox Proportional Hazards model was fitted to evaluate the strength of LNR compared to LN stage (within the NPI model) in both cohorts. The effect of clinicopathological variables on OS was analyzed using multivariable analysis in the Galway cohort. Three models were created (Model 1: Traditional prognostic variables excluding NPI and LNR; Model 2: Model 1 and LNR; Model 3: Model 1 and NPI) and compared using Likelihood-ratio tests.

Model

<table>
<thead>
<tr>
<th>Model</th>
<th>Variables</th>
</tr>
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<td>1</td>
<td>Age, Menopausal Status, Grade, Histological Subtype, Size, LN Stage, Menopause, Stage, Molecular subtype based on immunohistochemistry, ER, PR, HER2 status</td>
</tr>
<tr>
<td>2</td>
<td>Variables in Model 1 and LNR</td>
</tr>
<tr>
<td>3</td>
<td>Variables in Model 1 and NPI groups</td>
</tr>
</tbody>
</table>

Stepwise variable selection was used to identify the best model to create a prognostic index and performance of the two indices was evaluated using Receiver Operating Curves (ROC).

Results: Controlling for tumor size and histological grade, LNR is a stronger prognostic factor than LN stage in both the Galway (β values of 1.2 and 0.3 respectively) and ONCOPOOL (β values of 1.3 and 0.3 respectively) cohorts, with LNR rendering LN stage non significant (p=0.135) in the former. In the Galway cohort, separate comparisons of Model 2 and 3 with Model 1 demonstrated that traditional clinicopathological variables in addition to LNR
HER2 Expression Is the Major Risk Factor for Recurrence in pT1a-b,N0 Breast Cancer: A French Regional Population-Based Study of 671 Patients.

Rouanet P, Daures JP, Roger P, Mathieu A, Romieu G, Cretin J, Barneon G, Granier M, Maran-Gonzalez A, Thibault S, Boissiere F, Bibeau F. Val d’Aurelle Cancer Institute, Montpellier, France; Montpellier 1 University, Montpellier; France; CHU Nimes, Nimes, France; ONCO LR Regional Network, Montpellier, France

Background
To evaluate the prognostic impact of HER2 overexpression in patients with pT1a-b, node negative, breast cancers.

Patients and Methods
A population of 1127 patients whose diseases were staged as pT1a-b, pN0 and who were treated in the Languedoc-Roussillon (ONCO LR Southern French regional network) from 1999 to 2004, was identified. 95% of patients had conservative management, no patient received adjuvant trastuzumab, 5% received chemotherapy and 80% anti-estrogen endocrine therapy. HER2 status was retrospectively assessed by immunohistochemistry in 671 patients (122 pT1a/549 pT1b). Kaplan-Meier method was used to estimate disease-free survival (DFS). Cox proportional hazard models were used to determine associations between HER2 status and disease-free survival adjusting on variables significantly linked to it.

Results
9-year Overall survival was 95%. HER2 overexpression (3+) was observed in 5.2% of the patients (n=35). HER2 3+ category was most frequently identified in the following sub groups: pT1a lesion (12.3% vs 3.6%; p=0.0001), mastectomies (14% vs 4.4%; p=0.023), Grade 2-3 (91% vs 50%; p=0.0001), estrogen receptor (ER) (negative (-) tumors (57% vs 30%; p<0.0001), progesterone receptor (PR) – tumors (74% vs 42%; p=0.0002). HER2 3+ was less frequent with adjuvant hormonal treatment (43% vs 80%; p<0.0001), 33 relapse (5%) were observed with a median follow-up of 6.4 years (range, 0.3 to 9.9 years). The 5-year DFS rates were 78% and 95% in patients with HER2-positive and HER2-negative tumors, respectively (p=0.017).

According to the immunohistochemical phenotype DFS5 were 95%, 94%, 85%, 73.6% for ER+/PR+/HER2- (n=502/75%), ER+/PR-/HER2- (n=124/20%), ER+/PR+/HER2 3+ (n=15/2%) and ER-/PR-/HER2 3+ tumors (n=20/3%), respectively (p=0.02).

In univariate analysis, HER2 positive tumors (p=0.017), phenotype classification (p=0.02) and adjuvant treatment (p=0.013) were significant prognostic factors. In multivariate analysis, only patients with HER2 3+ tumors had higher risks of recurrence (hazard ratio [HR], 2.41; 95% CI: [1.06-5.53]; p<0.05) than those with HER2 - tumors.

Discussion
Node-negative, pT1a-b, breast cancer patients overexpressing HER2 have a significant risk of recurrence at 6 years median follow-up. In our series of small breast tumors, HER2 status seems to be a better prognostic factor than ER status. In patients with hormone receptor–positive diseases, HER2 positivity is associated with a worse DFS despite an anti-estrogen treatment.
P2-12-18
A Prognostic Model Based on Node Status, Cathepsin-D and Ki-67
Predict the Outcome of Patients Failing To Achieve Pathological
Complete Response after Anthracycline-Based Neoadjuvant
Chemotherapy for Breast Cancer.
Chen S, Chen C, Yu K, Shao Z. Shanghai Cancer Hospital, Fudan
University, Shanghai, China

Background: Aim of this study was to evaluate factors which
could possibly affect the outcome of patients failing to achieve pathological
complete response (pCR) after anthracycline-based neoadjuvant
chemotherapy (NCT) for breast cancer, and built a prognostic model
to predict disease free survival (DFS) and overall survival (OS).

Material and Methods: We retrospectively collected data of
199 stage II-III breast cancer patients who had failed to achieve
pathological complete response after neoadjuvant chemotherapy in
Shanghai Cancer Hospital. The NCT regimens were NE (nabvblbine
25mg/m2, day 1, 8 and epirubicin 60mg/m2, day 1; every three weeks)
or CEI (cyclophosphamide 600 mg/m2, day 1; epirubicin 60 mg/m2,
day 1 and 5-fluorouracil 600 mg/m2, day 1; every three weeks) for a
median treatment course of 3 cycles. To develop the prognostic model,
variables at baseline(age, menopausal status, tumor size, node status,
ER, PR, and HER-2) and at surgery(tumor size, grade, histotype,
node status, vascular invasion, ER, PR, HER-2, Cathepsin-D, P53,
Topo-IIα, Nm-23, Bcl-2, BAX, MDR, GSTn, PS2, P27, Cyclin D1
and Ki-67) were investigated. Multivariate Cox regression model
and Kaplan-Meier method was used to build the model, and show the
discrimination of DFS and OS in different risk groups.

Results: By multivariate analysis, ≥4 lymph node metastasis (HR=1.9,
P<0.009), Cath-D positive (HR=2.5, P=0.006), and Ki-67 index≥20%
(HR=1.9, P=0.001) at surgery were independent predictors for lower
DFS and OS rate among non-pCR responders. We built a prognostic
scoring model on the basis of these variables, in which ≥4+ nodes,
Cath-D + or Ki-67 index≥20% would contribute 1 point separately
to the risk score. The 5-year DFS rates in low(0 point), intermediate-
low(1 point), intermediate-high(2 points) and high risk groups(3 points)
were 94%, 65%, 43%, and 28%, respectively (Log-rank test p<0.001). The 5-year OS rates in these four groups were 94%, 84%,
66%, and 34%, respectively (Log-rank test p<0.001). Conclusion:
Post-NCT node status, Cathepsin-D and Ki-67 were used to develop a
prognostic model which could simply discriminate patients with poor
prognosis among non-pCR responders after anthracycline-
neoadjuvant chemotherapy, and might help to tailor further individualized treatment strategies.

P2-11-19
Nomogram To Predict Recurrence and To Avoid Unnecessary
Adjuvant Chemotherapy Based on Ki67 Index and ER Status
in Hormone Receptor (HR)-Positive Breast Cancers with Low
Number of Nodal Metastases (≤3) (NCT101273415).
Park YH, Im S-A, Cho EY, Ahn JH, Kim S, Keam B, Han W, Park IA,
Center; Seoul National University College of Medicine

Background: Hormone receptor (HR) positive breast cancers
characterized with ER-associated genes are differentiated luminal B
from luminal A tumors mainly by proliferation genes. According to
NCCN guideline 2011, node positivity has been a main determinant
to decide adjuvant chemotherapy with category 1. However, the
experts’ panel at the St. Gallen Consensus in 2009 do not provides
definite indications to give or withhold chemotherapy in patient
group with intermediate criteria including low numbers (1-3, N1)
of involved lymph nodes. Thus, in cases of limited number of nodal
metastases, the role of biologic factors including Ki67 index needs to
be defined. The aims of this study are to evaluate of Ki67 index as a
useful surrogate marker to predict recurrence and to avoid unnecessary
adjuvant chemotherapy and to develop nomogram based on Ki67
index to determine adjuvant therapeutic options in HR-positive in
N0 and N1 breast cancers.

Patients and Methods: We retrospectively analyzed the
clinicopathologic characteristics and outcomes of 953 postoperative
HR-positive N0 and N1 breast cancer patients between 2004 and 2007
at the Samsung Medical Center. We constructed nomogram based
on Cox regression model using independent factors demonstrated
in multivariate analysis and validated externally in a cohort of 895
patients treated at Seoul National University Hospital.

Results: In Cox regression multivariate analysis, ER–ve/PgR+ve and
Ki67 index were identified as independent factors.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Hazard Ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER positive/PgR positive</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>ER negative/PgR positive</td>
<td>0.016</td>
<td>2.646</td>
</tr>
<tr>
<td>Ki67 (categorical variable)</td>
<td>0.0001</td>
<td>0.000</td>
</tr>
<tr>
<td>0+</td>
<td>0.0001</td>
<td>0.000</td>
</tr>
<tr>
<td>1+</td>
<td>0.0114</td>
<td>0.047-0.286</td>
</tr>
<tr>
<td>2+</td>
<td>0.0076</td>
<td>0.287</td>
</tr>
<tr>
<td>3+</td>
<td>0.522</td>
<td>0.753</td>
</tr>
<tr>
<td>4+</td>
<td>0.317</td>
<td>1.826</td>
</tr>
</tbody>
</table>

Nomogram base on Cox-regression model showed an AUC of 0.75
(95% CI, 0.72-0.77) in the training set. The validation set showed a
good discrimination with an AUC of 0.63 (95% CI, 0.60-0.66). We
defined low nomogram score as less than 53, and high nomogram
score as 53 or more from the cut-off value of the nomogram ROC
curve. Patients who received anthracycline-containing adjuvant
chemotherapy with high nomogram scores showed better DFS with
statistical significance than those who did not receive anthracycline-
containing adjuvant chemotherapy with high nomogram scores
(p<0.0001). In contrast, the patients with low nomogram scores
did not show any benefit from anthracycline-containing adjuvant
chemotherapy (p=0.804). When the patients with high nomogram
scores divided into two groups according to Allred ER scores (0-4 vs
5-8), the patients with high ER Allred scores (5-8) and high nomogram
scores did not show any benefit from anthracycline-containing
chemotherapy (p=0.283). Main benefit from adjuvant chemotherapy
is focused on the patients with low ER Allred scores (0-4) and high
nomogram score (p=0.022).

Conclusion: Ki67 index is useful as a valuable surrogate marker
to predict recurrence and to avoid unnecessary chemotherapy.
Nomogram based on Ki67 index is constructed and validated to
determine adjuvant therapeutic options in HR-positive N0 and N1
breast cancers.

P2-12-20
Adjuvant! Online is Overoptimistic in Predicting Survival of
Asian Breast Cancer Patients.
Blaho Pathy N, Yip CH, Hartman M, Saxena N, Taib NA, Bulgiba AM,
vander Graaf Y, Verkooijen HM. University of Malaya; University
Medical Center Utrecht; Ministry of Health Malaysia; National
University of Singapore; Karlinska Institute

Background: Adjuvant! Online is a free web-based tool which
dicts 10-year breast cancer outcomes and efficacy of adjuvant
therapy in patients with breast cancer. As its prognostic performance
has only been validated in high income Caucasian populations, the
model was validated in a middle income Asian setting.

Material and Methods: Within the University Malaya Hospital-Based
Breast Cancer Registry, all 631 women receiving standard
surgery for invasive non-metastatic breast cancer between 1993 and
2000 were identified. Calibration of Adjuvant! Online was evaluated
Adjuvant! Online performed fairly in terms of discrimination, with an area under ROC curve of 0.73 (95% CI: 0.69-0.77).

Conclusion: Even though Adjuvant! Online is capable of discriminating between good and poor survivors, it systematically overestimates survival. These findings suggest that the model requires adaptation prior to use in Asian settings.

### P2-12-21

**Impact of Recent Parity on Histopathological Tumor Features and Outcome of Young Women with Breast Cancer.**

Nagatsuma AK, Shimizu C, Tsuda H, Saji S, Hojo T, Sugano K, Fujiwara Y. National Cancer Center Hospital; Satama Medical University International Medical Center; Tochigi Cancer Center Research Institute

**Background:** Younger age has been associated with worse outcome in breast cancer patients (pts) and recent parity has been epidemiologically identified as worse prognostic factor among women with breast cancer. The objective of this study was to explore potential factors associated with worse prognosis in young breast cancer pts, and to demonstrate the impact of parity on the histopathological tumor feature and patient outcome.

**Materials and Methods:** We retrospectively analyzed 634 early breast cancer pts younger than 45 years old who underwent surgery between 2000 and 2009. For statistical analysis, Pearson’s and Fisher’s exact test were used. Survival analysis was performed only for pts diagnosed before 2006 in order to obtain a minimum follow up 5 years. **Results:** 108 women were diagnosed within 5 years since last parity (Group A), 216 were diagnosed > 5 years since last parity (Group B) and 310 were nulliparous (Group C). Median age at diagnosis was 37 (range 26-44), 41 (range 32-44), and 38.5 (range 22-44) and family history (FH) of breast and/or ovarian cancer within second degree was found in 23, 22, and 23% of the pts in Groups A, B, and C, respectively. In Groups A, B and C, clinical stage was III in 22, 10 and 12% (p = .025), ER was positive in 65, 69 and 70% (p = .650), PgR was positive in 64, 75 and 74% (p = .057), and HER2 was positive in 25, 14 and 14% (p = .017), respectively. In Group A had higher histological grade (grade 3: 60/44/47%, p = .019), higher nuclear grade (grade 3: 61/47/48%, p = .036) and more lymph vessel invasion (61/52/45%, p = .015) compared to those in Groups B and C, respectively. Median follow up time was 85.1 months (range 1.8-137.1 months) during which there were 61 deaths. In univariate analysis, age and FH were not correlated with overall survival (OS). OS in Group A was significantly lower than in Group B (hazard ratio (HR) 3.51, 95% confidential interval (CI) 1.80-6.84, p<.001) and in Group C (HR 2.42, 95% CI 1.36-4.29, p=.002), while OS did not differ significantly between Groups B and C. In the pts without FH, the HR of cancer death was more pronounced in Group A than in Group B (HR 4.25, 95% CI 1.97-9.14, p<.001) or Group C (HR 2.67, 95% CI 1.43-5.01, p=.002), while there was no significant difference among the groups in pts with FH. In multivariate analysis among the pts without FH, lymph vessel invasion (HR 4.51, 95% CI 1.89-10.76, p=.001), Group A women (HR 2.28, 95% CI 1.25-4.17, p=.007), histological grade 3 (HR 2.72, 95% CI 1.28-5.77, p=.009), PgR negativity (HR 2.23, 95% CI 1.19-4.18, p=.013) and clinical stage II and III (HR 2.92, 95% CI 1.04-8.21, p=.04) were significantly associated with poor prognosis, adjusting for age.

**Conclusion:** Recent parity was associated with worse histopathological features in breast cancer of women younger than 45. It was also associated with worse outcome, especially among pts without FH. Recent parity seems to be a confounding factor for the worse outcome in young breast cancer patients, which justifies further studies to elucidate underlying biology.

### P2-12-22

**A Prognostic Index Composed of Progesterone Receptor Status, Tumour Size and S-phase Fraction, Predicts Survival in Node-Negative Breast Cancer Patients in a Large Multicentre Prospective Cohort Study.**

Klintman M, Nilsson F, Bendahl P-O, Fernö M, Liljegren G, Emdin S, Malmström P. Clinical Sciences, Lund University and Skane University Hospital, Lund, Sweden; University of Umeå and Umeå University Hospital, Umeå, Sweden; Örebro University Hospital, Örebro, Sweden

**Background:** The importance of the added prognostic value of proliferation, either single factors such as Ki67 or flow cytometric S-phase fraction (SPF), or as the main common denominator in the majority of gene expression profiles, has been highlighted over the last years in node-negative breast cancer. There are however few published prospective studies. In an earlier retrospective study on node-negative breast cancer from our group, a prognostic index consisting of PR status, tumour size and a proliferation factor, SPF, identified one third of the patients as high risk, with a fourfold increased risk of distant recurrence. The present study was set up to validate this index in a large prospective multicenter cohort study with long term follow-up.

**Material and Methods:** 596 patients from 3 regions in Sweden were between 1991-1995 included in the study. Inclusion criteria were: 10mm≤tumour size≤50mm, node-negativity, ≥5 lymph nodes
removed, age <60 years, and radical surgery. Patients with bilateral breast cancer or previous malignancy were excluded. Prospective analyses of ER, PR, and flow cytometric SPF were performed. High-risk was defined as 2 or more of the following: 1. size ≥20mm 2. PR- (in the absence of PR status, ER-) and 3. high SPF (in the absence of SPF, Bloom Richardson grade 3). 82% of the patients received no adjuvant medical treatment. Cox proportional hazards regression, stratified for centre, was used to model the impact of the index on breast cancer specific survival (BCSS). Median follow-up was 16 years for the 452 patients alive at last follow-up. Analyses were done after 5 and 10 years follow-up, during which 42 and 95 patients, respectively, died of breast cancer. Results: 31% of the patients were identified as high-risk. In univariate analysis, the index was prognostic for BCSS after 5 years (HR 5.1, 95%CI: 2.7-9.8) as well as 10 years (HR 2.2, 95%CI: 1.5-3.4). The prognostic impact remained significant after adjustment for adjuvant medical treatment and age. The 5- and 10-year BCSS (95%CI) was 97% (94-98) and 87% (83-90) for low risk patients, compared with 85% (79-89) and 76% (69-81) for high risk patients. In the group with no risk factors (n=218), no patient died of breast cancer during the first 5 years. Proliferation was the strongest factor for BCSS followed by PR and tumour size, both in uni- and multivariate analyses. In multivariate analysis, adjusted for adjuvant treatment and age, the HR for proliferation was 7.5 (95%CI: 3.2-18) after 5 years and 2.5 (95%CI: 1.6-4.0) after 10 years. Discussion: This large prospective multicenter cohort study validates the results from an earlier retrospective study, that a prognostic index consisting of PR status, tumour size and a proliferation factor, SPF, reliably identifies one third of the node-negative patients with a high risk of relapse. The index also identifies an extreme low risk group; patients with no risk factors, with 100% 5-year survival. This group could be spared adjuvant medical treatment, especially chemotherapy. Taken together, this index may be clinically helpful for prognostic considerations and for selection of adjuvant treatment.

P2-12-23
How Should We Assess Tumour Size (T Stage) in Patients with Multicentric/Multifocal Breast Cancer? Results from the NCIC CTG MA.5 Randomized Trial of CEF vs. CMF in Pre-Menopausal Women with Node Positive Breast Cancer.

Bougamin N, Dong B, Hilton JF, Chapman J-AW, Arnaud A, O’Malley F, Nielsen T, Gelmon K, Verushalmi R, Levine M, Bramwell V, Whelan T, Pritchard KJ, Shepherd L, Clemons M, The Ottawa Cancer Center; Ottawa, ON, Canada; Queens University, Kingston, ON, Canada; The Ottawa Hospital, Ottawa, ON, Canada; Mount Sinai Hospital-University of Toronto, Toronto, ON, Canada; Vancouver Hospital and Health Sciences Center, Vancouver, BC, Canada; BC Cancer Agency-University of British Columbia, Vancouver, BC, Canada; Juravinski Cancer Center-McMaster University, Hamilton, ON, Canada; Tom Baker Cancer Center-University of Calgary, Calgary, AB, Canada; Odette Cancer Center-University of Toronto, Toronto, ON, Canada

Background: A common clinical conundrum in breast cancer management is whether pathologic T stage in women with multicentric or multifocal disease should be taken as the diameter of the largest focus or as the sum of all foci in the breast. Most staging systems, such as the American Joint Committee on Cancer (AJCC), simply use the largest tumour focus for staging. We examine here the impact of alternate methods of estimating tumour size including measures of total tumour size, volume and surface area.

Methods: NCIC CTG MA.5 is a randomized trial of CEF versus CMF in pre-menopausal women with node positive breast cancer. Median follow up is 10 years. Pathologically reported patient tumour dimensions for up to 3 foci were utilized to examine the effects of tumour size on Breast-Cancer-Free-Interval (BCFI). BCFI is defined as the time from randomization until recurrence: first local invasive or DCIS, regional, distant, contralateral invasive or DCIS. Tumour size was estimated as 1) pathologic T stage as per AJCC criteria; 2) largest dimension of largest tumour focus (cm); 3) sum of largest dimension(s) of tumour foci (cm); 4) sum of surface area(s) of tumour foci (cm²), and 5) sum of volume of tumour foci (cm³). Step-wise forward unstratified Cox regression was used to assess the different effects of tumour size.

Results: This study accrued 710 patients, 37% with T1 tumours, 52% with T2 tumours and 9% with T3 tumours; 61% had 1 to 3 positive lymph nodes. 59% hormone receptor positive. Higher pathologic T stage (p=0.001) and greater surface area (p=0.02) were associated with shorter BCFI, as was lymphovascular invasion (p=0.03), and # of lymph nodes involved (p=0.0001). Administration of anthracycline therapy led to significantly longer BCFI (0.003). The sum of largest tumour sizes (p=0.33) and sum of tumour volume (p=0.34) were not significantly associated with BCFI. Additionally, when the less complete locally reported tumour grade data were included, higher tumour grade was associated with shorter BCFI (p=0.0001).

Conclusions: Consideration of multicentric and multifocal disease was an important adjunct to standard pathologic tumour size as was estimation of tumour surface area in this chemotherapy trial of node positive premenopausal women. However, simply adding together the diameters of tumours in patients with multicentric or multifocal disease did not add any additional prognostic information in this high risk patient population.

P2-12-24
Resumption or Persistence of Menstruation after Cytotoxic Chemotherapy Is a Poor Prognostic Factor for Disease Free Survival in Premenopausal Patients with Early Breast Cancer.

Park IH, Han H-S, Lee KS, Kang HS, Lee S, Kim SW, Jung SY, Shin KH, Ro J. National Cancer Center, Goyang, Korea; Chungbuk National University, Korea

Background: We investigated the relationship between resumption or persistence of menstruation after chemotherapy and disease free survival (DFS) in premenopausal patients with early breast cancer.

Patients and methods: A total of 843 patients diagnosed with a stage I to III breast cancer between March 2001 and December 2006 were included in this study. All patients received cytotoxic chemotherapy after surgery; 411 (48.8%) with anthracycline based, 416 (49.3%) with anthracycline and taxane containing, and 16 (1.9%) with other regimens. We reviewed the medical records with a long term follow-up.

Results: The median age of patients was 41 years (range, 21-54 years) and the median follow-up duration was 6.2 years (range, 0.7-10.4 years).
Table 1. Patient characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. of patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (median yr, range)</td>
<td>47 (21-54)</td>
</tr>
<tr>
<td>Hormone receptor</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>632 (75%)</td>
</tr>
<tr>
<td>Negative</td>
<td>211 (25%)</td>
</tr>
<tr>
<td>HER2 receptor</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>133 (15.8%)</td>
</tr>
<tr>
<td>Negative</td>
<td>415 (49.2%)</td>
</tr>
<tr>
<td>Not known</td>
<td>255 (33%)</td>
</tr>
<tr>
<td>Stage</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>209 (24.8%)</td>
</tr>
<tr>
<td>II</td>
<td>458 (55%)</td>
</tr>
<tr>
<td>III</td>
<td>189 (22.4%)</td>
</tr>
<tr>
<td>Not known</td>
<td>7 (0.8%)</td>
</tr>
<tr>
<td>Body Mass Index (BMI)</td>
<td></td>
</tr>
<tr>
<td>&lt;18.5kg/m2</td>
<td>37 (4.4%)</td>
</tr>
<tr>
<td>18.5-22.9kg/m2</td>
<td>451 (51.1%)</td>
</tr>
<tr>
<td>≥23kg/m2</td>
<td>374 (44.4%)</td>
</tr>
<tr>
<td>Missing</td>
<td>1 (0.1%)</td>
</tr>
<tr>
<td>Adjuvant chemotherapy</td>
<td></td>
</tr>
<tr>
<td>Anthracycline based</td>
<td>411 (48.8%)</td>
</tr>
<tr>
<td>Taxane containing</td>
<td>416 (49.3%)</td>
</tr>
<tr>
<td>Others</td>
<td>16 (1.9%)</td>
</tr>
<tr>
<td>Adjuvant hormonal therapy</td>
<td></td>
</tr>
<tr>
<td>CIA</td>
<td>621 (73.7%)</td>
</tr>
<tr>
<td>Present</td>
<td>658 (78.1%)</td>
</tr>
<tr>
<td>Absent</td>
<td>102 (12.1%)</td>
</tr>
<tr>
<td>Not known</td>
<td>83 (9.8%)</td>
</tr>
<tr>
<td>Resumption of period</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>442 (52.4%)</td>
</tr>
<tr>
<td>No</td>
<td>403 (47.6%)</td>
</tr>
</tbody>
</table>

Of all, 632 (75%) patients were hormone receptor (HR) positive who received tamoxifen therapy upon completion of chemotherapy. The chemotherapy induced amenorrhea (CIA) rate was 78.1% (n=658) and 52.4% (n=442) experienced the resumption of the long term follow-up. One hundred two (12.1%) patients had persistent menstruation without CIA. The disease free survival (DFS) was significantly affected by the young age (≤35 years) (HR=1.58, [95% CI, 1.10-2.70], P=0.014), advanced stage (stage 3) (HR=4.45, [95% CI, 3.17-6.25], P<0.001), HER negativity (HR=2.21, [95% CI, 1.57-3.12], P<0.001), HER2 positivity (HR=1.56, [95% CI, 1.04-3.24], P=0.032), and the resumption or persistent period (HR=2.03, [95% CI, 1.42-2.91], P<0.001). HR negativity (HR=2.03, [95% CI, 1.43-2.90], P<0.001), advanced stage (HR=4.40, [95% CI, 3.12-6.21], P<0.001), and the resumption or persistent period (HR=1.85, [95% CI, 1.24-2.77], P=0.002) were remained significant factors for DFS on multivariate analysis.

Table 2. Analysis for disease free survival in all patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age ≤35 years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.58 (1.10-2.70)</td>
<td>0.014</td>
<td>1.07 (0.71-1.60)</td>
</tr>
<tr>
<td>Resumed/persistent period</td>
<td></td>
<td></td>
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<tr>
<td>2.03 (1.42-2.91)</td>
<td>0.001</td>
<td>1.35 (1.24-2.77)</td>
</tr>
<tr>
<td>Stage III vs. I/II</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.45 (1.77-3.41)</td>
<td>0.001</td>
<td>2.40 (1.32-4.31)</td>
</tr>
<tr>
<td>HR negativity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.21 (1.57-3.12)</td>
<td>0.001</td>
<td>2.03 (1.43-2.90)</td>
</tr>
<tr>
<td>HER2 positivity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.56 (1.04-2.34)</td>
<td>0.032</td>
<td>1.18 (0.78-1.79)</td>
</tr>
<tr>
<td>BMI &gt;23kg/m2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.66 (0.24-1.78)</td>
<td>0.412</td>
<td></td>
</tr>
<tr>
<td>BMI &lt;18.5 kg/m2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.05 (0.75-1.48)</td>
<td>0.767</td>
<td></td>
</tr>
</tbody>
</table>

Conclusions

A considerable proportion of premenopausal patients treated by chemotherapy experienced resuming period after CIA. The resumption or persistence of menstruation was a poor prognostic factor for disease free survival in premenopausal patients with early breast cancer.

P2-12-25
Blinded Multi-Site Validation of a Pathology-Based Prognostic Marker Profile for Operable Hormone Receptor-Positive Breast Cancer.
Linke SP, Bremer TM, Man AK, Bloom KJ, Lawton TJ. Prediction Sciences, La Jolla, CA; Clarient, Inc., Aliso Viejo, CA; Seattle Breast Pathology Consultants, Seattle, WA

Background: We previously reported development and preliminary validation of a marker profile with a risk of recurrence algorithm for patients with operable hormone receptor (HR)-positive breast cancer using tissue microarrays. The profile includes ER, PR, HER2, EGFR, BCL2, and p53 (assessed by IHC) and MYC/Bq24 (assessed by FISH). It also directly incorporates the standard clinicopathologic risk factors tumor size, tumor grade, and nodal status to the extent that their prognostic value is not replaced by the molecular markers. Here, we demonstrate validation of the profile in a blinded multi-site study conducted on whole sections.

Material and methods: The study was conducted in a blinded fashion using an independent data management firm (Synteract, Carlsbad, CA). Eligible patients were females diagnosed between 1985 and 1997 with HR-positive stage I-IIIA breast cancer treated only with hormone therapy after definitive surgery for whom sufficient tumor samples were available for testing. Slides with FFPE tumor tissues and associated clinicopathologic, treatment, and outcome data were provided by four clinical sites through the NCI-funded Cooperative Breast Cancer Tissue Resource program: Fox-Chase Cancer Center (Philadelphia, PA) (n=106), Kaiser Permanente Northwest (Portland, OR) (n=165), University of Miami (FL) (n=55), and Washington University St. Louis (MO) (n=74). Marker assays, scoring, and calculation of risk scores using a predefined algorithm were conducted at Clarient, a CLIA-certified laboratory. The algorithm assigned a risk score to each patient on a scale of 0 to 10+, and a predefined threshold of 3.8 was used to separate patients into low and high risk groups.

Results: Complete data for all seven markers was obtained in 349 of the 400 patients. The algorithm defined 28% of the patients (n=99) as high-risk and 72% of the patients (n=250) as low-risk. The high-risk and low-risk patient groups had 10-year distant metastasis rates of 34% and 9%, respectively, resulting in a hazard ratio of 4.7 (95% CI, 2.3-7.8, p<0.0001) through 10 years without an intermediate-risk group. Similar results were achieved when using disease-specific survival (death with evidence of breast cancer recurrence) as the outcome. Using a standard threshold score of 3.4, the Nottingham Prognostic Index (NPI) also stratified the patients (n=349) into low and high risk groups (p=0.03), but only 10% of the patients were in the low-risk category, and the profile further risk stratified patients in the NPI intermediate and high risk categories similar to the overall patient set (p<0.001). In multivariate Cox proportional hazards analysis with clinicopathologic factors, only the profile and tumor size were significant (Wald statistic p=0.001 for both).

Discussion: This blinded study, using a predefined algorithm and threshold, validates the prognostic and clinical utility of the multi-marker profile to help guide the appropriate level of adjuvant treatment in breast cancer patients.
P2-12-26

Rezai M, Eiermann W, Kümmel S, Kühn T, Warm M, Friedrichs K, Schneeweiss A, Markmann S, Eggemann H, Hilfrich J, Jackisch C, Witzel I, Eidmann H, Kaufmann M, Blohmer JU, Lüsenkranzhaus, Düsseldorf, Germany; Rotkreuzklinikum, München, Germany; Klinikum Essen-Mitte, Essen, Germany; Klinikum Esslingen, Esslingen, Germany; Krankenhaus Holweide, Köln, Germany; Brustzentrum, Hamburg, Germany; Universitätsklinikum, Heidelberg, Germany; Universitätsklinikum, Rostock, Germany; Universitätsklinikum, Magdeburg, Germany; Eilenriedeklinik, Hannover, Germany; Klinikum Offenbach, Offenbach, Germany; Universitätsklinikum Eppendorf, Hamburg, Germany; Universitätsklinikum, Kiel, Germany; Universitätsklinikum, Frankfurt, Germany; Sankt Gertrauden-Krankenhaus, Berlin, Germany

Background: Oncotype DX® has become part of clinical routine in the diagnosis and decision-making progress in early breast cancer (EBC). Prospective data on its clinical use and impact on treatment decisions in ER-positive (ER+) node negative (N0) disease from various controlled clinical trials in different countries have been published recently. The Recurrence Score® (RS) has also been validated as a prognostic and predictive marker for patients with ER+ node positive (N+) disease. As of today no prospective data have been reported on its impact on decision making in these patients. We performed a large prospective study to evaluate RS-guided adjuvant therapy in N0 and N+ ER+ EBC.

Material and Methods: Patients (pts) with ER+, HER2-negative N0 and N+ (1-3 positive lymph nodes) EBC and no contraindication for adjuvant chemotherapy were included in the study. Physicians’ adjuvant treatment recommendations and their confidence in these as well as patients’ decisional conflicts were assessed before and after knowledge of the results of the test using standardized questionnaires. Actual treatment data were collected to perform pharmacoeconomic analyses. Analyses were performed on the per-protocol population for whom a Recurrence Score result and treatment recommendations pre and post-test were available.

Results: Overall 379 pts were recruited. In 11 pts Oncotype DX could not be performed and 2 pts dropped out leaving 366 pts in the per protocol population. Of these, 244 (66.7%) were N0 and 122 (33.3%) N+. Median age was 56 years (Range 25-85). Overall, 54.1% had low, 38.0% intermediate and 7.9% high RS values. For N0 disease, the distribution of RSs was 53.7%, 38.9%, 7.4% and for N+ disease 54.9%, 36.9% and 9.0%, respectively.

Initial treatment recommendation changed in 33.1% of all cases; 30.3% in N0 and 38.5% in N+ disease, and in 36.4% for pts with low, 30.9% with intermediate and 20.7% with high RSs. In 21.6% of all pts a recommendation for adjuvant chemotherapy was changed from HT to CHT. For N0 disease change rates were 18.4% from CHT to HT and 11.5% from HT to CHT. For N+ disease 27.9% of recommendations changed from HT to CHT and 9.0% of recommendations changed from CHT to HT. In 25% of all, 22% of N0 and 39% of N+ pts initially recommended HT the post-RS recommendation changed to CHT; in 32% of all, 39% of N0 and 37% of N+ pts initially recommended CHT the recommendation changed to HT.

Overall, physicians’ confidence increased in 45.1% of all (p=0.047) and 44.7 of N0 and 45.9% of N+ cases, respectively. There was a moderate decrease of the decisional conflict score in all pts and subgroups that reached statistical significance for all pts (p=0.029) and the low RS subgroup (p=0.003).

Conclusions: Results of this large prospective study show an impact of the RS on adjuvant treatment decision making in German clinical practice for patients with ER+ EBC. Recommendations were predominantly changed from chemoendocrine to endocrine adjuvant therapy resulting in a net reduction of chemotherapy usage. This effect was more pronounced for patients with 1-3 positive nodes.

P2-12-27
Simply Adding Together the Diameters of Tumor Foci in Patients with Multicentric or Multifocal Disease Does Not Add Any Additional Prognostic Information: An Analysis from NCIC CTG MA.12 Randomized Placebo-Controlled Trial of Tamoxifen after Adjuvant Chemotherapy in Pre-Menopausal Women with Early Breast Cancer.

Hilton JP, Dong B, Bouganin N, Chapman J-AW, Arnaout A, OMalley F, Nielson T, Gelmon K, Yerushalmi R, Levine M, Bramwell V, Whelan T, Priehardt KI, Shepherd L, Clemmons M, Queens University, Kingston, ON, Canada; The Ottawa Hospital, University of Ottawa, Ottawa, ON, Canada; Mt. Sinai Hospital, University of Toronto, Toronto, ON, Canada; Vancover Hospital and Health Sciences Centre, University of British Columbia, Vancouver, BC, Canada; BC Cancer Agency, University of British Columbia, Vancouver, BC, Canada; Juraviski Cancer Centre, McMaster University, Hamilton, ON, Canada; Tom Baker Cancer Centre, University of Calgary, Calgary, AB, Canada; Odette Cancer Centre, University of Toronto, Toronto, ON, Canada

Background: A common clinical conundrum in breast cancer management is whether pathologic T stage in women with multicentric or multifocal disease should be taken as the diameter of the largest focus or as the sum of all foci in the breast. Most staging systems, such as the American Joint Committee on Cancer (AJCC), simply use the largest tumor focus for staging. We examine here the impact of alternate methods of estimating tumour size including measures of total tumor size, volume and surface area.

Materials & Methods: NCIC CTG MA.12 is a randomized placebo-controlled trial of tamoxifen after adjuvant chemotherapy for pre-menopausal women with early breast cancer. Median follow up is 9.7 years. Pathologically reported patient tumor dimensions for up to 3 foci were utilized to examine the effects of tumor size on Breast-Cancer-Free-Interval (BCFI), defined as the time from randomization until recurrence (defined as first local, regional, distant, or contralateral invasive tumor or DCIS). Tumor size was estimated as 1) pathologic T stage as per AJCC criteria; 2) largest dimension of largest tumor focus (cm); 3) sum of largest dimension(s) of tumor foci (cm²); 4) sum of surface area(s) of tumor foci (cm²), and 5) sum of volume of tumor foci (cm³). Step-wise forward unstratified Cox regression was used to assess the different effects of tumor size.

Results: This study accrued 672 patients, 43% with T1 tumors, 51% with T2 tumors, and 6% with T3/T4 tumors; 25% were node negative and 56% had 1-3 positive lymph nodes. 75% were locally determined to have hormone receptor positive tumors. A higher number of involved lymph nodes was associated with significantly shorter BCFI (p<0.0001). None of pathologic T stage (p=0.14), largest dimension of largest tumor size (p=0.14), sum of largest dimension(s) of tumor foci (cm); 4) sum of surface area(s) of tumor foci (cm²), and 5) sum of volume of tumor foci (cm³). Step-wise forward unstratified Cox regression was used to assess the different effects of tumor size.

Discussion: In the MA.12 population of pre-menopausal women randomized to either tamoxifen or placebo, the sole factor significantly
associated with BCFI was nodal status. No measure of tumor size in unifocal or multicentric/multifocal tumors impacted BCFI. The findings of this mature data set suggest that simply adding together the diameters of tumors in patients with multicentric or multifocal disease did not add any additional prognostic information.

P2-12-28
Studies of a Malignancy-Associated Protein, Osteopontin, in NCIC CTG MA.14, a Randomized Trial of Tamoxifen Versus Combined Tamoxifen and Octreotide LAR in Adjuvant Treatment of Women with Early Breast Cancer.
Bramwell VH, Tuck AB, Chapman J-A, Anborgh PH, Postenka CO, Shepherd L, Pritchard KJ, Han L, Wilson C, Pollak M, Chambers AF. Tom Baker Cancer Centre, Calgary, AB, Canada; London Regional Cancer Program, London, ON, Canada; NCIC Clinical Trials Group, Kingston, ON, Canada

Background: We have shown, in women with metastatic breast cancer (BC), that high baseline plasma osteopontin (OPN) levels and increases over time are associated with poor survival. In primary BC, increased immunohistochemical (IHC) expression of OPN in tumour is associated with poorer survival and is elevated in lymph node metastases relative to primary tumour. Our current study evaluates tumour and baseline/serial plasma OPN levels after resection of primary BC.

Methods: In NCIC CTG MA.14, 667 postmenopausal women post surgery received 5 yrs Tamoxifen (T) +/- 2 yrs monthly Octreotide LAR. There were no differences in survival outcomes between arms. OPN was assayed by IHC in primary BC. OPN was measured in plasma at baseline (up to 4 mos post randomization) and serially in recurrent (18 mos time window) versus non-recurrent cases, by ELISA. Statistical Analysis: IHC and plasma OPN had Box-Cox variance stabilization transformations (power 0.5; logarithm). Descriptive characterization of both OPN measurements is provided for all patients, by baseline patient and tumour characteristics, and by recurrence status. Univariate stratified log-rank statistics and hazard ratios with 95% CI were generated for intention to treat (ITT), event for free survival (EFS), relapse fee survival (RFS), overall survival, and for other clinical and pathological potential covariates by Cox regression was used, with factor added if p<0.05.

Results: From 667 MA.14 patients, 647 patients were assessed for OPN: 462 (69%) by IHC; 387 (58%) at baseline in plasma (with 2,542 samples total from serial sampling), IHC % tumor positivity had mean 33.9% (95% CI 30.2%-37.9%), while baseline plasma OPN was mean 46.1 (44.6-47.6) ng/ml. In 80 women with recurrence, mean OPN during the recurrence window was 60.7 (52.8-69.8) ng/ml. Neither baseline IHC or plasma OPN levels were significantly associated with any of the endpoints in either univariate or multivariate analyses. Older age (p=0.02), greater nodal involvement (p=0.001), higher baseline body mass index (p<0.0001), and higher tumour grade (p=0.0001) were multivariately associated with shorter EFS. Baseline plasma OPN was not correlated with IHC OPN (Pearson 0.02, p=0.73).

Discussion: Mean baseline OPN plasma level 46.1 ng/ml (median 45 ng/ml) in MA.14 was similar to our previous findings in healthy women (median 47 ng/ml). In contrast, we previously showed that 63% of 158 women with a new diagnosis of metastatic BC had elevated OPN levels with median value 177 ng/ml. Our results thus far suggest that tumor and plasma levels, while associated with prognosis in metastatic BC, may not be so in primary BC following surgery. However, in MA.14, the 80 women with recurrent BC had significantly higher mean OPN in the recurrence window, 60.7 ng/ml, than at baseline. We will present further analyses exploring this finding.

P2-12-30
Pre-Operative Haematological Markers and Prognosis in Early Breast Cancer.
Cordiner RL, Mansell J, Obondo C, Angerson WJ, Lannigan A, McMillan D, Wilson CR, Doughty JC, Western Infirmary, Glasgow, United Kingdom; Wishaw General Hospital, Wishaw, United Kingdom

Background: Systemic inflammation may influence survival in women with breast cancer. Both the platelet lymphocyte ratio (PLR) and the neutrophil lymphocyte ratio (NLR) have been shown to be associated with survival in a number of solid tumours. The aim of this study was to assess the role of PLR and NLR in survival of women with early breast cancer.

Methods: Women diagnosed with early breast cancer at two centres between 2003 and 2006 were included. All women had measured white cells, neutrophils, lymphocytes and platelets. NLR and PLR were calculated. Cox regression survival analysis was performed with breast cancer specific survival used as the primary end point. Significant associations of NLR and PLR with pathological variables was assessed using the Chi squared test.

Results: 707 women were included with a median follow up of 3.7yrs. On univariate analysis neither NLR (HR 1.08, 95% CI 0.66-1.79) or PLR (HR 0.96, 95% CI 0.58-1.59) were significantly associated with breast cancer specific survival. An increased PLR was found to be associated with significantly fewer grade 1 tumours (P<0.05) but no other significant associations with pathology was demonstrated for either PLR or NLR.

Conclusions: This study would suggest that the NLR and PLR are not prognostic of survival in women with early breast cancer.

P2-12-31
Moderate Immunohistochemical Expression of HER2 (2+) without HER2 Gene-Amplification Is a Negative Prognostic Factor in Early Breast Cancer.
Rossi V, Sarotto I, Maggiorettro F, Tomasi Cont N, Redana S, Aglietta M, Ponzone R, Montemurro F, Institute for Cancer Research and Treatment IRCC, Candiolo, Turin, Italy

Purpose
We sought to evaluate whether moderate HER2 immunohistochemical (IHC) expression (2+ and no HER2/amplification) identifies early breast cancer (EBC) with a distinct prognostic profile.

Methods
A total of 1295 women (median age 58, range 22-94) undergoing surgery for EBC from Jan 1995 to Sept 2009 were retrospectively reviewed. All patients had undergone HER2 testing by the HercepTest and, when needed, by fluorescence in-situ hybridization (FISH). All tests were carried out at our Institutional Surgical Pathology laboratory. The impact of HER2 status on disease-free survival (DFS) was corrected for other clinical and pathological potential covariates by Cox Proportional Regression Analysis.

Results
A total of 494 (38%), 486 (38%), 119 (9%) and 196 (15%) of the patients had HER2 0+, 1+ 2+ and positive (3+ or FISH+) tumors, respectively. Sixty-one of 196 patients with HER2-positive EBC received adjuvant trastuzumab. A total of 298 DFS events occurred at median follow-up of 59 months (4-137 months). By using HER2 0+ status as reference, multivariate analysis revealed that HER2 2+ expression was associated with a significant increase in the risk of
a DFS event (HR 2.303, 95% CI 1.501-3.533, p<0.001), whereas HER2 1+ was not (HR 0.976, 95% CI 0.698-1.365, p=0.889). We performed exploratory two-group comparisons by further classifying tumors according to hormone-receptor status (cutoff for ER and PgR positivity ≥10% of stained cells). Results are summarized in the table. HER2 2+ expression was associated with worse prognosis in both ER and/or PgR positive and ER/PgR negative tumors. In the latter group, HER2 2+ expression was associated with a particularly high rate of DFS events, with a 60-month projected DFS of 20%. Furthermore, while DFS curves plateaued at 60 months for HER2-positive tumors (with and without adjuvant trastuzumab) and for ER/PgR negative tumors with HER2 0/1+ expression, hormone receptor positive/HER2 2+ tumors displayed a particularly high rate of late relapses (beyond 60 months). Results did not change using a 1% cutoff to define ER and PgR positivity.

Conclusion

Moderate HER2 positivity (IHC 2+/FISH negative) identifies EBC and PgR positivity.

Her2 2+ tumors displayed a particularly high rate of late relapses (beyond 60 months). Results did not change using a 1% cutoff to define ER and PgR positivity.

P2-12-32

Association between Progranulin (GP88) Expression and Recurrence Risk for Breast Cancer Patients with Estrogen Receptor Positive Invasive Ductal Carcinoma.

Serrero G, Hawkins DM, Joffe O, Bejarano P, Phillips JT, Head JF, Elliott RL, Godwin AK, Weaver J, Yue B. A&G Pharmaceutical Inc, Columbia, MD; University of Minnesota, Minneapolis, MN; University of Maryland, Baltimore, MD; University of Miami, Miami, FL; EEH Breast Cancer Research and Treatment Center, Baton Rouge, LA; University of Kansas Medical Center, Kansas City, KS; Fox Chase Cancer Center, Philadelphia, PA.

Purpose:

GP88 (progranulin) is a critical player of breast tumorigenesis for estrogen receptor positive (ER+) breast cancer. Pathological studies showed that GP88 was expressed in invasive ductal carcinoma (IDC), but not in normal mammary tissue, benign lesions or lobular carcinoma. The present study examines GP88 prognostic significance in association with recurrence risk for patients with ER+ IDC.

Patients and Methods:

Two retrospective multi-site clinical studies examined GP88 expression by immunohistochemistry (IHC) analysis in paraffin-embedded tumor tissues in correlation with patients’ survival outcomes. The training study established a GP88 cut-off value associated with decreased disease-free (DFS) and overall (OS) survivals. The validation study verified the GP88 cut-off value and compared GP88 prognostic information with other prognostic factors in multivariate analysis.

Results:

GP88 expression is associated with a statistically significant increase in recurrence risk for ER+ IDC patients. The training study established that GP88 3+ score by IHC analysis was associated with decreased DFS (p=0.0004) and OS (p=0.0036). The independent validation study verified that GP88 3+ score for the high risk group and demonstrated that GP88 3+ score was associated with a 5.9-fold higher hazard of disease recurrence and a 2.5-fold higher mortality hazard compared to patients with tumor GP88≤3+. GP88 remained an independent risk predictor after considering age, nodal status, tumor size, tumor grade, progesterone receptor expression, treatment and disease stage.

Conclusion:

Our training and validation studies demonstrate that the survival factor GP88 is a prognostic biomarker, predictive of recurrence risk and increased mortality for ER+ IDC patients, independent from other prognostic factors. These results provide support for measuring GP88 tissue expression for newly diagnosed early stage breast cancer patients.

This work was supported by grants R43CA124179, and U01CA113916 from the National Cancer Institute, grants 07-2007-064 and 02-2010-01 from the Avon Foundation for Women.

P2-12-33

Withdrawn by Author

P2-13-01

Gene Profiling of Whole Blood May Identify Patients with BRCA Mutations.

Mina LA, Gokmen-Polar Y, Goswami C, Storniolo AM, Li L, Badve S, Sledge GW. Indiana University School of Medicine, Indianapolis, IN.

Background: The BRCA1 and the BRCA2 proteins play a role in DNA repair and confer genomic stability to the cell. Identifying BRCA mutation carriers has become an important tool for prevention as well as guiding therapy in cancer patients. We proposed to test the hypothesis that gene expression analysis of peripheral whole blood can reliably detect these mutations.

Materials and methods:

Following IRB approval, 10cc of blood was collected from 36 women (BRCA1 (n=8), BRCA2 (n=9), Hereditary breast cancer without BRCA (FAM) (n=7), sporadic breast cancer (SPO) (n=11)). 3 of BRCA1 and 5 of BRCA2 samples were from women without cancer. Following RNA extraction (using the method described by Beckman et al) and quality assessment, Illumina® Whole-Genome DASL™ microarray (Human Ref-8 BeadChips) analysis was performed. The raw data was normalized and analyzed using Partek® Genomic Suite. Differentially expressed genes were identified using ANOVA analysis. Geneset specific supervised analysis was performed to visualize the inherent similarities and differences in the gene expression amongst different groups for 1) DNA repair and 2) Immune-system-related genes. Ingenuity Pathway Analysis (IPA) was performed to interpret the data in the context of biological processes, pathways and networks.

Results:

Twenty-nine of the 87 immune-related genes were up-regulated in BRCA1 and BRCA2 groups compared to SPO or FAM groups; these included IL7R, CD53, CD2, CD48 and HLA-DRA. Twenty-five of the 79 DNA repair genes were up-regulated in BRCA1 and BRCA2; these included FANCC, RAD51L3, MSH2, MSH6 and PCNA. In IPA analysis, the comparison of BRCA1 vs. REST (BRCA2 + FAM + SPO) showed a strong immunologic signal, with the top altered biological processes including “Immunologic disease”, “Infection mechanism”, “Immune cell trafficking” and “cell-mediated immune response”. The top 5 canonical pathways also reflected a similar pattern and included “ICOS-ICOSL Signaling in T Helper Cells”, “OX40 Signaling Pathway”, “Calcium-induced T Lymphocyte”, “Apoptosis Regulation of IL-2 Expression in Activated and Anergic...”
T Lymphocytes” and “Protein Ubiquitination Pathway”. When BRCA2 was compared with the REST (BRCA1 + FAM + SPO), a much weaker signal was noted with none of the canonical pathways being significantly altered. PAM analysis showed that a set of 16 genes could differentiate the BRCA patients from the rest with an error rate of 5%. Further validation of this genaset is being performed.

Conclusion:
Gene profiling in whole blood may offer an easy, reliable and inexpensive way to identify patients with BRCA mutation. Further studies are currently underway to validate our results in a larger patient population.

P2-13-02
Parent of Origin of BRCA Mutation May Determine Age at Breast Cancer Diagnosis.
Shapira I, Budman DR, Akerman M, Weiselberg L, Vinciguerra V, D’Olimpio J, Devoe C, Cheng KL, Donahue L, John V, Cohen S. Hofstra North Shore LIJ School of Medicine, Lake Success, NY; Feinstein Institute for Medical Research, Manhasset, NY

Background
Genetic diseases may display parent-of-origin effects. In such cases, the risk depends on the specific parent or origin allele. Imprinting effect is evident in autosomal dominant hereditary paraganglioma leads to tumors only if inherited from paternal germline. Cancer penetrance in mutations carriers may be determined by the parent origin of BRCA mutation.

Methods
From 2007-2010 we analyzed 1889 consecutive (136 ovarian + 1753 breast) breast (BrCa) or ovarian cancer (OvCa) patients presenting for treatment at our outpatient facility. In 130 patients with BRCA 1 or 2 mutations the parent of origin for the mutation was known. Of the 130 patients 2 had both BRCA1 and BRCA2 mutated paternal inherited and were excluded from this analysis. Of the breast cancer patients: 28 patients had paternal and 29 had maternal BRCA1 mutations, 24 had paternal and 21 had maternal BRCA2 mutations. Of the ovarian cancer patients 6 had paternal and 10 had maternal BRCA1 mutations, 7 had paternal and 3 had maternal BRCA2 mutations. In carriers of BRCA mutations the mean age at diagnosis for ovarian cancer was 51 (range 21-70) and for breast cancer was 43 (range 24-78).

Two-sample t-test was used to compare the mean age at diagnosis in patients with BRCA 1 or 2 mutations of paternal or maternal inheritance.

For breast cancer maternal allele versus paternal allele 2-sample t-test and p-value were compared for the age at first diagnosis. For breast cancer patients BRCA1 maternal inheritance (mean±SD yrs) 45.73±11.22 versus paternal inheritance 38.04±7.14 2-sample t-test p-value p<0.0020. For breast cancer BRCA2 maternal inheritance (mean±SD yrs) 50.65±10.44 versus paternal inheritance 41.68±6.16, 2-sample t-test p-value p<0.0008.

Results:
Significantly younger age at breast cancer diagnosis was observed in paternal vs. maternal inheritance of BRCA1 mutation (38 vs 46, respectively, p=0.0020) and BRCA2 mutation (42 vs 51 respectively, p<0.0008). There was no significant difference between paternal and maternal age of ovarian cancer diagnosis of BRCA1 (p=0.1415) or BRCA2 mutation (p<0.3470).

Conclusion:
The restrospective nature of the study may introduce ascertainment bias. However, the breast and ovarian cancers cases in BRCA1 & 2 carriers with maternal or paternal inheritance mirror the Mendelian autosomal dominant pattern in our unselected consecutive cohort of patients. Maternal and paternal inherited BRCA alleles may not be exchangeable. Women with paternally inherited mutations in BRCA gene mutations develop breast cancer at younger age compared with women who inherit the gene mutations from their mothers. In this small sample, clear differences at age of cancer diagnosis are apparent in paternal inheritance of BRCA gene mutation. If this observation duplicates in larger cohorts results will have important implications for recommendation of surgical risk reduction in BRCA mutation carriers.

P2-13-03
Beattie MS, Ganschow P, Gabram-Mendola S, Wilson A, Joseph G, Lee R, Loranger K, Stanislav C, Seelaus C, Farrell R, Trim L, DelPozo S, Luce J. University of California, San Francisco, CA; San Francisco General Hospital, San Francisco, CA; Stroger Hospital of Cook County, Chicago, IL; Rush University Medical Center, Chicago, IL; Emory University, Atlanta, GA; Grady Memorial Hospital, Atlanta, GA

Background: Underserved women at risk for Hereditary Breast and Ovarian Cancer (HBOC) are confronted with many unique challenges, such as barriers to accessing appropriate genetic testing and counseling services and decreased resources, that place them increased cancer risk. It is unclear whether these high-risk women are appropriately referred for genetic counseling and what their genetic test results demonstrate due to minimal practice-based evidence. To study this population, and to establish an infrastructure to further explore long-term outcomes, we formed the Consortium of Underserved BRCA testers in October 2010 from: San Francisco General Hospital (SF), Stroger Hospital of Cook County (Chicago), and Grady Memorial Hospital (Atlanta).

Methods: Using common clinical and research protocols and mixed methods analysis, we examined and compared referral patterns, demographics, and BRCA test results between sites. We used chart reviews and common data collection instruments to gather and pool data. Using descriptive and comparative statistics, we examined similarities and differences between Consortium sites.

Results: SF’s program began 9 years ago, Chicago’s began 6 years ago, and Atlanta’s began 3 years ago. Medicaid funding for BRCA testing has been available in SF since 2011, in Chicago since 2009, and is not yet available in Atlanta. P values were all <0.05 for comparing referral sources between sites. Each site uses unique referral tools and systems. For example, in SF, a family history screening questionnaire is administered during mammography; in Chicago, an extensive primary care network has been educated to refer patients for genetic counseling and what their challenges, such as barriers to accessing appropriate genetic testing and counseling services and decreased resources, that place them at increased risk of breast cancer. It is unclear whether these high-risk women are appropriately referred for genetic counseling and what their genetic test results demonstrate due to minimal practice-based evidence. To study this population, and to establish an infrastructure to further explore long-term outcomes, we formed the Consortium of Underserved BRCA testers in October 2010 from: San Francisco General Hospital (SF), Stroger Hospital of Cook County (Chicago), and Grady Memorial Hospital (Atlanta).

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Beattie MS, Ganschow P, Gabram-Mendola S, Wilson A, Joseph G, Lee R, Loranger K, Stanislav C, Seelaus C, Farrell R, Trim L, DelPozo S, Luce J. University of California, San Francisco, CA; San Francisco General Hospital, San Francisco, CA; Stroger Hospital of Cook County, Chicago, IL; Rush University Medical Center, Chicago, IL; Emory University, Atlanta, GA; Grady Memorial Hospital, Atlanta, GA

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Table 1: Referrals to Genetic Counseling and Testing in the Consortium of Underserved BRCA Testers.

<table>
<thead>
<tr>
<th>Referral Source</th>
<th>SF</th>
<th>Chicago</th>
<th>Atlanta</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of referrals</td>
<td>1341</td>
<td>1510</td>
<td>91</td>
</tr>
<tr>
<td>Breast clinic</td>
<td>17%</td>
<td>18%</td>
<td>55%</td>
</tr>
<tr>
<td>Oncology</td>
<td>16%</td>
<td>16%</td>
<td>21%</td>
</tr>
<tr>
<td>Mammography</td>
<td>16%</td>
<td>25%</td>
<td>19%</td>
</tr>
<tr>
<td>Primary care</td>
<td>20%</td>
<td>17%</td>
<td>N/A</td>
</tr>
<tr>
<td>Family members</td>
<td>5%</td>
<td>N/A</td>
<td>1%</td>
</tr>
<tr>
<td>Other (University, community, private, emergency department, and self-referrals)*</td>
<td>14%</td>
<td>N/A</td>
<td>3%</td>
</tr>
<tr>
<td>*p &lt; 0.05 in chi-squared analysis</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Cancer Res; 71(24 Suppl.) December 15, 2011 304s Cancer Research
Table 2 demonstrates similar BRCA positive rates (13-18%) despite statistically significant differences in race/ethnicity between sites.

<table>
<thead>
<tr>
<th>Site</th>
<th>BRCA Testers</th>
<th>Chicago</th>
<th>Atlanta</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of BRCA Testers</td>
<td>260</td>
<td>375</td>
<td>70</td>
</tr>
<tr>
<td>Caucasian</td>
<td>37%</td>
<td>38%</td>
<td>47%</td>
</tr>
<tr>
<td>African American</td>
<td>31%</td>
<td>29%</td>
<td>29%</td>
</tr>
<tr>
<td>Asian</td>
<td>10%</td>
<td>6%</td>
<td>2%</td>
</tr>
<tr>
<td>Other</td>
<td>5%</td>
<td>5%</td>
<td>5%</td>
</tr>
</tbody>
</table>

*Includes Native American, Pacific Islander, and Middle Eastern

Conclusions: Despite differences in referral patterns and Medicaid funding, underserved women at all 3 public hospital sites had similar BRCA positive rates, which are in line with positive rates from University Cancer Centers. We believe this relates to the availability of genetic counseling services and a similar testing threshold at each site. The large and ethnically diverse population of this Consortium can serve as a valuable resource for researchers and clinicians. We plan to follow this cohort prospectively to study clinical outcomes and medical decisions after BRCA testing in underserved families at risk of HBOC.

**P2-13-04**


Turco DL, Elsayegh N, Litton J, Hortobagyi GN, Arun B. M.D.

Anderson Cancer Center, Houston, TX

Background: Infiltrating lobular carcinoma (ILC) is the second most common type of invasive breast cancer behind infiltrating ductal carcinoma (IDC). ILC makes up approximately eight percent of all invasive lesions. In addition, the mixed ductal and lobular carcinoma histology makes up approximately seven percent of all invasive diagnoses.

There are several biologic differences that are demonstrated by ILC compared to other common pathologies. They are more frequently bilateral and multicentric. They tend to be seen in women slightly older than the average age of diagnosis and are usually ER positive. It is also known that individuals with a genetic predisposition have an increased risk to develop breast cancers. Women with a mutation in their CDH1 gene have up to a 39% chance to develop ILC. The majority of hereditary breast cancer is caused by germline mutations in the BRCA1 and BRCA2 genes. Women with a mutation in their BRCA1 or BRCA2 gene have up to an 87% risk to develop an invasive breast cancer, however, the presence of ILC in this population has not been well defined. Therefore, the aim of this study is to evaluate the rate of germline BRCA mutations in a cohort of patients both with pure ILC, as well as mixed ILC/IDC.

Methods: A retrospective chart review revealed one hundred and sixty nine women with ILC and mixed ILC/IDC who underwent genetic testing for mutations in the BRCA1 and BRCA2 genes through the Clinical Cancer Genetics Program at M. D. Anderson Cancer Center. Women were referred for genetic testing using referral guidelines based on the NCCN guidelines, this usually involves a personal history of early onset breast cancer and/or a family history of breast and/or ovarian cancer.

Results: Out of the 169 patients, 19 (11.24%) were found to have a germline mutation in their BRCA1 or BRCA2 gene. A significant majority (73.7%) of these patients were BRCA2 positive. Five women tested for a variant of uncertain significance. The average age of diagnosis for the cohort was 55.6 years (range 30-87); while the average age of diagnosis for a positive patient was 49.4 years (range 30-72). Of the 62 women with pure ILC, 5 (8.06%) were positive for a BRCA gene mutation. Historically, out of all the patients with breast cancer referred and tested through the Clinical Cancer Genetics Program, approximately 15% test positive, and research shows that in the general population, 7-10% of breast cancer patients will test positive.

Conclusions: while the positivity rates between the cohorts are not statistically significant, we have shown that patients with a BRCA mutation can develop ILC in addition to the more commonly seen IDC. We suggest that patients continue to be referred for genetic counseling according to the NCCN guidelines, regardless of the pathology of their tumor.

**P2-13-05**

Breast Cancer, BRCA Mutations and Attitudes Regarding Pregnancy and Preimplantation Genetic Diagnosis.

Litton JK, Etzel CJ, Jackson MA, Muse KI, Turco D, Schover LR, Theriault RL, Mattair D, Lu KH, Hortobagyi GN, Arun BK. The University of Texas MD Anderson Cancer Center, Houston, TX

Background: Breast cancer is associated with treatment-related infertility and has been demonstrated to be a major concern for premenopausal survivors. Detection of a BRCA deleterious mutation may also affect attitudes regarding future childbearing. Preimplantation genetic diagnosis (PGD) allows women to use in vitro fertilization (IVF) to implant only those embryos without a BRCA mutation. The ability to test the fetus for BRCA mutations is also available through amniocentesis and chorionic villus sampling (CVS). The objective of this study was to evaluate attitudes about childbearing and fertility in women being evaluated for a BRCA mutation. Methods: Women with childbearing potential who were referred to the Clinical Cancer Genetics Clinic to be evaluated for a BRCA mutation were invited to participate in this survey. The questionnaire was administered prior to genetic counseling. A follow-up was administered after the BRCA results were disclosed. The survey queried participants regarding their attitudes on fertility, pregnancy as it may relate to cancer and the potential of a BRCA mutation. Other questions detailed attitudes regarding IVF, PGD, and CVS in these instances. Descriptive statistics were used. Results: One hundred and twenty-eight women completed pre-questionnaires and to date 76 have completed post results disclosure questionnaires. The mean age was 33 (range 21-44) with 69.5% with a diagnosis of breast cancer, 39.8% received chemotherapy and 60.9% already had at least 1 biological child. A future child was desired by 45.3% although 53.1% worried that their children would have an increased risk of cancer. Regarding PGD, although only 30.9% (38/123) said that they would use PGD, 80.2% felt that the testing should be available to families with inherited cancers. Regarding fetal testing via amniocentesis or CVS, 29.7% would have the fetus tested and 7% would consider termination if a genetic mutation was identified. Additionally, 69.5% felt it was important to receive fertility counseling and treatment at the same place where they receive their cancer care. To date 8 women have been diagnosed with a BRCA1 mutation and 4 with a BRCA2 mutation. When asked similar questions after their genetic results were disclosed, 2 women who had previously stated they would not use PGD changed their mind. Conclusions: Future pregnancies are important to many breast cancer survivors. BRCA mutation carriers have the option to have children without passing on their genetic risk for cancer. Although few would use these interventions, a large majority felt it was important to have information about these choices and to have options for fertility preservation options addressed at the


Background: Patients with BRCA1-BRCA2 germline mutation who developed a first breast cancer (BC) have a high risk to develop a second BC, especially if aged less than 40 at first diagnosis. Thus, a positive BRCA test may influence the therapeutic management and orient towards non-conservative surgery to reduce new BC occurrence. We set up a fast-track process to get BRCA1/2 and sometimes p53 mutation results within the shortest possible interval, in recently diagnosed BC patients whose probability to be mutation carriers was high (>20%).

Methods: Between 05/02/2009 and 03/01/2011, 61 patients diagnosed with localized BC requesting neo-adjuvant or adjuvant chemotherapy and whose personal or familial history fulfilled BRCA1/2 or P53 genetic testing criteria, were proposed genetic counselling. After a first multidisciplinary team meeting (MT), genetic testing was proposed and performed according to usual genetic counselling rules. Psychological support was offered at every step of the process. Patients were informed that their test would be analysed as part of a fast-track process and were aware of the potential consequences of a positive test. Results were communicated and discussed during a second MT, when initial therapeutic plan was eventually changed.

Results: All women who were referred to the genetic clinic for fast-track testing accepted the analysis. The mean age at diagnosis was 38 (range 26-55). The whole process (from 1st genetic consultation till disclosure consultation) was completed in a median interval of 99 days (range 19-245). All pts received appointment within 5 days, when results were available. 50% pts postponed for more than 15 days their disclosure appointment (range 0-113 days). One patient (pt) declined result disclosure. 18 pts (30%) were found to be carriers of a deleterious mutation (13 BRCA1, 4 BRCA2, 1 p53). Variants of unknown significance (UV) in BRCA1 or BRCA2 genes were identified in another 4 patients. In 18 cases, surgery type initially proposed was changed because of the presence of the germline mutation: all pts were proposed ipsilateral mastectomy eventually associated to immediate reconstruction as often possible, as well as contralateral mastectomy. Final surgery turned to bilateral mastectomy in 3 patients with deleterious BRCA mutation and in one suspect UV. 14 patients with mutation accepted the change, but four BRCA2 and one BRCA1 mutation carriers refused mastectomy or bilateral mastectomy. Radiotherapy was cancelled in a p53 mutation carrier.

Conclusion: This data show that genetic testing might be considered as part of therapeutic decision. Moreover, 22% (4/18) opted for a simultaneous contralateral prophylactic mastectomy. On the other hand, some women did not change their mind with mutation knowledge (4/18, 22%). Psychological impact of this procedure is currently retrospectively assessed by a self-questionnaire, fulfilled by mail. Analyses will be available for the meeting.

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Nb</th>
<th>Treatment changes between 1st and 2nd MT</th>
<th>Change accepted by patient</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRCA1 mutation</td>
<td>13</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>BRCA2 mutation</td>
<td>4</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>P53 mutation</td>
<td>1</td>
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</tr>
</tbody>
</table>

Prevalence of Germline BRCA1 and BRCA2 Deleterious Mutations in Brazilian Patients with High-Risk of Breast and Ovarian Cancer.

Diz MDPE, Escobar KA, Guindalini RSC, Pasini FS, SnitkowskI IMI, Maistro S, Hoff PMG, Federico MHII, Instituto do Cancer do Estado de Sao Paulo, Sao Paulo, Brazil; Faculdade de Medicina da Universidade de Sao Paulo, Sao Paulo, Brazil

Background: Germline mutations in the BRCA1/2 genes account for the majority of hereditary breast ovarian cancer (HBOC). Distribution and prevalence of clinically relevant mutations in BRCA1/2 differ among different populations. All of the Brazilian studies published to date have not performed complete sequencing of both genes, restricting their analyses to selected exons. In addition, a highly heterogeneous mutation spectrum was observed. The lack of data concerning the prevalence and profile of BRCA1/2 deleterious mutation and the appropriated screening criteria for DNA sequencing in the Brazilian population complicates the design of screening strategies.

Objective: To accurately determine the contribution of BRCA1/2 germline mutations in a well characterized sample of Brazilian breast and ovarian high-risk pts.

Methodology: A total of 73 unrelated pts attending a Brazilian public hospital from September 2005 to July 2006, with HBOC risk ≥10% according to Frank, Evans or BRCAPRO algorithms, underwent complete sequencing and MLPA analysis of BRCA1/2 genes. Demographic data and tumor pathological features were extracted from prospectively registered medical records.

Results: Mean age, median of cancer cases in families and median score by Frank, Evans and BRCAPRO algorithms were respectively 48y (24-78), 4 (0-13), 11.2 (0-56.3), 13.4 (0,1-100) and 16 (2-75). A total of 79 unknown clinical significance mutations were identified: 44 in BRCA1 and 35 in BRCA2. Known deleterious mutations were identified in 8 pts. Four in BRCA1: R71G at exon 5, 5382insC at exon 20 (2 patients) and R1751X at exon 20; and four in BRCA2: R2318X at exon 13, R3128X at exon 25, 5844del5 and 663del5 at exon 11. Several polymorphisms were found in all pts. No mutation was detected by MLPA technique. One novel frameshift deleterious mutation in BRCA2: 6610insTT at exon 11 which results in a stop codon was detected in a breast cancer patient and in four of her relatives from a family with high-risk of HBOC. Taking into account the novel mutation, the prevalence of BRCA1/2 mutation in high-risk pts in our cohort is 12.3%. Pts characteristics presented in [Table 1].

Conclusion: To the best of our knowledge, the study represents the first report of the complete sequencing and MLPA analysis of BRCA1/2 genes in large Brazilian series of HBOC high-risk pts. The prevalence of germline mutations found in the present study was similar to the reported by other studies examining high-risk populations.

Characteristics of patients with BRCA1/2 deleterious mutations

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Age of BC diagnosis</th>
<th>Histology</th>
<th>HR status</th>
<th>HER2 status</th>
<th>BRCA1/2</th>
<th>Frank</th>
<th>Evans</th>
</tr>
</thead>
<tbody>
<tr>
<td>B2: R3128X</td>
<td>41</td>
<td>IDC</td>
<td>pos</td>
<td>pos</td>
<td>0,493</td>
<td>26,3</td>
<td>22</td>
</tr>
<tr>
<td>B2: 5384del5</td>
<td>35</td>
<td>IDC</td>
<td>pos</td>
<td>pos</td>
<td>0,05</td>
<td>10,2</td>
<td>22</td>
</tr>
<tr>
<td>B2: 6610insTT</td>
<td>45</td>
<td>IDC</td>
<td>pos</td>
<td>ind</td>
<td>0,16</td>
<td>17,5</td>
<td>10</td>
</tr>
<tr>
<td>B2: R2318X</td>
<td>44</td>
<td>N/C</td>
<td>N/C</td>
<td>N/C</td>
<td>0,44</td>
<td>30,8</td>
<td>31</td>
</tr>
<tr>
<td>B2: R3632G</td>
<td>42</td>
<td>IDC</td>
<td>pos</td>
<td>pos</td>
<td>0,903</td>
<td>20</td>
<td>22</td>
</tr>
<tr>
<td>B1: 5382insC</td>
<td>64</td>
<td>IDC</td>
<td>neg</td>
<td>neg</td>
<td>0,31</td>
<td>16,4</td>
<td>31</td>
</tr>
<tr>
<td>B1: R4582X</td>
<td>43</td>
<td>IDC</td>
<td>pos</td>
<td>pos</td>
<td>0,493</td>
<td>26,3</td>
<td>22</td>
</tr>
<tr>
<td>B1: 3582insC</td>
<td>53</td>
<td>IDC</td>
<td>neg</td>
<td>ind</td>
<td>0,764</td>
<td>31,8</td>
<td>31,8</td>
</tr>
</tbody>
</table>

B1=BRCA1; B2=BRCA2; BC=breast cancer; IDC=Invasive ductal carcinoma; MC=medullary carcinoma; ILC=invasive lobular carcinoma; HR=Hormone receptor; (b)=bilateral, N/A=not available; N/C=never had cancer; pos=positive; neg=negative; ind=indeterminate
P2-13-08

Kwong A. Chu ATW. The University of Hong Kong, Hong Kong; The Hong Kong Hereditary Breast Cancer Family Registry, Hong Kong

Background: Research in the West has shown that the uptake rates of surveillance and prophylaxis may be different in BRCA mutation carriers and non carriers and this may vary between different ethnicities. To date there is limited comprehensive data available for the behavioural pattern of Asian cohorts. The present on-going study is the first to investigate the behavioural impact and medical compliance of Chinese high risk females who had undergone BRCA1/2 genetic testing up to 1.5 years after genetic testing.

Methods: 88 females with personal or family breast and/or ovarian cancer history who had BRCA1/2 genetic testing performed by the Hong Kong Hereditary Breast Cancer Family Registry were surveyed by a face-to-face or telephone interview. Sociodemographic information, genetic test results, pre-and post-testing surveillance practice, chemoprevention methods used and attitudes towards clinical management were obtained. Retrieval of medical records was performed using a prospective database.

Results: 39 carriers and 49 noncarriers of a BRCA 1/2 mutations were interviewed. 82.1% of carriers and 79.6% noncarriers had breast and/or ovarian cancer prior to testing. The uptake rate of breast surveillance by eligible carriers (85.2%) and noncarriers (71.1%) remained high after knowing the test results. 12.8% and 15.4% of the eligible carriers had prophylactic contralateral mastectomy and salpingo-oophorectomy respectively, while 5.1% chose to have both procedures within 1.5 years of knowing the genetic test result. Significantly more carriers (85.7%) than noncarriers (49%) underwent ovarian surveillance (p=0.004). 25.6% of carriers and 26.5% of noncarriers with breast cancer history chose Western chemoprevention methods, while no carriers without breast cancer history chose such methods. 34.7% of carriers (30.8% of noncarriers) used Chinese herbal medicine as complementary prevention treatment after knowing the genetic test result. Clinicians’ advice was perceived as the major reason of choosing a particular surveillance or preventative strategy.

Conclusions: As population-based breast screening is unavailable in Hong Kong, the relatively high uptake rate of breast screening among noncarriers may be the result of enhancement in health-consciousness due to prior personal or family cancer experience. Proper education by clinicians is crucial to increase uptake of high risk screening. Traditional Chinese herbal medicine methods are commonly used as a form of prevention. A longitudinal prospective study will contribute to the understanding of attitude-change towards clinical management of this Chinese population who may have different cultural beliefs.

P2-13-09
First Statewide Experience with Telephone Delivery of Cancer Genetic Counseling.


Background: National guidelines and multiple professional medical societies recommend genetic counseling as the standard of care for women with a personal and/or family history suggestive of a hereditary cancer syndrome. In addition, the ability to provide risk assessment and genetic counseling is a requirement for accreditation of breast centers by organizations such as the National Accreditation Program for Breast Centers (NAPBC) and the Commission on Cancer. However, there is clear evidence that this standard is not being achieved. Many individuals are undergoing genetic testing for these syndromes without adequate pre- and post-test counseling and, on the other hand, many individuals who are appropriate for genetic testing are not being identified and referred for genetic counseling and therefore not receiving potentially life-saving genetic information and services. A number of factors have been identified as contributors to these poor public health outcomes, including a lack of available genetic counseling services due to a limited number of trained genetics professionals and their limited geographic distribution. Research has demonstrated the effectiveness of cancer genetic counseling delivered by telephone, but to date, almost no real-world experience has been reported. We provided statewide access to board-certified genetic counselors via telephone in Michigan beginning in February 2010 and report our experience with the first 200 patients at risk for hereditary breast and ovarian cancer.

Materials and Methods: Baseline data were analyzed for all Michigan patients undergoing telephone genetic counseling through Informed Medical Decisions since service delivery began in February 2010. Variables include demographics, geographic location, personal cancer history, family cancer history, Gail risk and test appropriateness criteria.

Results: Analysis has been completed on the first 200 patients referred for consideration of hereditary breast and ovarian cancer. Outcomes include testing, results and, where applicable, reasons testing was not pursued, patient and provider satisfaction, as well as trends over time in patient volumes, providers using the service and uptake by geographic location.

Conclusion: Based on statewide experience in Michigan, telephone cancer genetic counseling is a viable method for service delivery in the real world. The Michigan experience can serve as a cost-effective model for improving genetic services in other locations with limited access to genetic experts.

P2-13-10
Detection, Visualization and High Resolution Physical Mapping of Large Rearrangements by Molecular Combing in the Hereditary Breast Cancer Genes BRCA1 and BRCA2.


The BRCA1 and BRCA2 genes are involved, with high penetrance, in breast and ovarian cancer susceptibility. About 2% to 4% of breast cancer patients with a positive family history who are negative for BRCA1 and BRCA2 point mutations can be expected to carry large genomic alterations (deletion or duplication) in one of the two genes, and especially BRCA1. However, large rearrangements are missed by direct sequencing.

Molecular Combing is a powerful FISH-based technique for direct visualization of single DNA molecules, allowing the entire genome to be examined at high resolution in a single analysis. We have developed a novel genetic test based on Molecular Combing. For that purpose, we designed specific BRCA1 and BRCA2 “Genomic Morse Codes” (GMC), also covering the non-coding regions and including large genomic portions flanking both genes. We developed a measurement strategy for the GMC signals, and validated our approach by blindly testing 10 breast cancer patients with a positive family history and 10 control patients. Large rearrangements, corresponding to deletions and duplications of one or several exons and with sizes ranging from 3 kb to 40 kb, were detected on both genes, including the characterization
of 4 new mutations (for BRCA1: Del ex 3, Del ex 24 and Dup ex 3; for BRCA2: Dup ex 17-20). The identified mutations confirmed the results obtained with high-resolution zoom-in aCGH (11 k) in the same patients, with a resolution in the 1-2 kb range. Importantly, the developed GMC allowed to unambiguously localize several tandem repeat duplications on both genes, and to precisely map large rearrangements in the problematic Alu-rich 5’-region of BRCA1.

We propose the developed Molecular Combing genetic test as a valuable tool for the screening of tandem repeat duplications, CNVs, and other complex rearrangements in BRCA1 and BRCA2, such as translocations and inversions, to be combined in clinical settings with an essay that allows the detection of point mutations. We see the main application of the developed molecular diagnostic tool as a predictive genetic test. However, we envisage to extended the application of the developed tool as a companion diagnostic test, for instance in the screening of BRCA1-mutated cells in the context of the development of PARP inhibitors. Thus, the genetic test may be applied not only to clinical blood samples, but also to circulating cells and heterogeneous cell populations, such as tumor tissues.

P2-13-11
Follow Up of BRCA1/2 Carriers: The Spectrum of Cancer Diagnoses in Healthy at Risk Individuals (HTR), and in Cancer Survivors (CS).

Introduction and Objective: Data from long term follow up of BRCA1/2 carriers is scarce and is mainly related to BRCA1 women. Our multidisciplinary program targets both women and men for counselling towards BRCA1/2 screening and inclusion in clinical follow up. In here we review all cancer diagnoses observed in our BRCA1/2 cohort during follow up.

Methods: Review of individual records of BRCA1/2 carriers registered from January 2000 to December 2010. Follow up was calculated since BRCA1/2 post-test counselling until the last visit to the Clinic. All new cancer diagnoses and preventive surgeries were registered.

Results: Two-hundred and fifty nine BRCA1/2 carriers (206 females and 53 males) were diagnosed with BRCA1/2 mutations (42 BRCA1 and 217 BRCA2). Medium follow up for all population is 25 months (1-98). At the date of initial BRCA1/2 diagnosis 99 women and 14 men were CS.

Female population: Eighty-eight female CS had been previously diagnosed with breast cancer (18 bilateral cases), 18 with ovarian cancer, and 1 with biliary tract cancer. Preventive surgeries in the CS female population were: bilateral adnexectomy (33 pts) and prophylactic contralateral mastectomy (10 pts). In this CS female population, new cancers, during follow up were: Contralateral breast cancer (4 cases), peritoneal cancer (2 cases in pts with previous prophylactic surgery) and skin non-melanoma cancers (2 cases). In female HTR, 23 preventive bilateral adnexectomies and 20 bilateral mastectomies were performed. Cancer diagnoses during follow up were: breast (11), peritoneum (1 in pt with previous prophylactic surgery), gastric (1) and M3 leukemia (1). Global failure of prophylactic adnexectomy, so far (CS+HTR): 3/56 (5%).

Male population: The medium age for male CS is 73 yrs and for male HTR is 52 yrs. Male CS had mostly been previously diagnosed with BC (12; 4 bilateral) and prostate cancer (4). Other previous cancers: gastric (2), skin (2: 1Melanoma, 1 non-Melanoma), colorectal (1). One BRCA2 man with gynecomasia and prostate cancer was submitted to reduction mastectomy, as a preventive surgery. During follow up, we diagnosed second and third cancers in male CS: breast (2), prostate (6) and gastric (1). Only 1 male HTR was diagnosed with cancer: skin non-melanoma.

Conclusion: The proportion of second and third cancer diagnoses in the male BRCA2 CS population is higher than in the female BRCA1/2 CS population. Small numbers and the availability of preventive surgery for women influence this observation. No data from preventive mastectomy exists for males (we have one case). The low frequency of cancer in male HTR may be due to younger age and other unknown modifier factors. Longer follow is needed.

P2-14-01
Race, Response to Chemotherapy, and Outcome within Clinical Breast Cancer Subtypes.
Tichy JR, Deal AM, Anders CK, Carey LA. UNC, Chapel Hill, NC

Introduction: Racial disparity in breast cancer (BC) outcome has been attributed to access to care, socioeconomic factors, and biologic factors. In particular, the prevalence of triple negative BC is significantly higher among African-American (AA) women than white women (non-AA). However a recent population-based study suggests that racial disparities persist even if stratified by subtype, although it is possible that bias in stage at presentation may have influenced these results. In this study, we examined 464 women of uniform clinical stage II-III breast cancer, all treated with neoadjuvant chemotherapy, and examined pathologic complete response (pCR), recurrence-free survival (RFS), and overall survival (OS) to see if racial disparity exists within a more homogeneous stage and treatment cohort. Methods: The UNC Neoadjuvant Breast Cancer Database is a prospectively maintained cohort with clinical and outcome annotation. We identified women diagnosed between 1991-2011 and assigned BC subtype by clinical assay for estrogen receptor and progesterone receptor (hormone receptors, HR) and HER2. Fisher’s Exact and Wilcoxon Rank sum tests compared characteristics between AA and non-AA. The Kaplan-Meier method and Log Rank test compared OS and RFS curves.

Results: 142 (31%) of 464 women were AA, 294 (63%) were white, and 28 (6%) were other races (for analysis white and “other” were categorized as non-AA). 191 (41%) were clinical stage II and 273 (59%) stage III at diagnosis. Stage at diagnosis did not differ between AA and non-AA (p=0.07). AA were slightly older (median age 49 v 45, p=0.01) and were more likely to be HR-/Her2- (44% v 25%, p=0.003). All patients received preoperative chemotherapy. AA received less biologic therapy such as trastuzumab or on-protocol bevacizumab (10% v 18%, p=0.03) and received significantly less endocrine therapy (46 v 59%, p=0.01) than non-AA patients. 102 (22%) of patients had a pCR to neoadjuvant chemotherapy; pCR rates did not differ significantly by race (p=0.7) or within subtype by race (all p>0.14). At a median follow-up of 4.5 years, 20% (126) of patients have died. Among HR-/HER2- (n=114) and HR-/HER2+ (n=47) patients, OS and RFS did not differ between AA and non-AA (all p>0.5). Conversely, among HR+/HER2- patients (n=159) AA had worse OS (p=0.01) and RFS (p=0.03) and among HR+/HER2+ patients (n=53), AA had worse OS (p=0.0004) and RFS (p=0.005).

<table>
<thead>
<tr>
<th></th>
<th>3 yr RFS</th>
<th>HR-/HER2</th>
<th>HR-/HER2</th>
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</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>142</td>
<td>102</td>
<td>40</td>
<td>26</td>
<td>26</td>
</tr>
<tr>
<td>HR+/HER2</td>
<td>36</td>
<td>28</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>AA</td>
<td>41% (25%-63%)</td>
<td>55% (43%-68%)</td>
<td>42% (31%-53%)</td>
<td>36% (25%-55%)</td>
<td>35% (22%-50%)</td>
</tr>
<tr>
<td>Non-AA</td>
<td>35% (25%-45%)</td>
<td>57% (47%-66%)</td>
<td>52% (41%-63%)</td>
<td>51% (38%-64%)</td>
<td>51% (35%-66%)</td>
</tr>
<tr>
<td>p-value</td>
<td>0.01</td>
<td>0.03</td>
<td>0.03</td>
<td>0.03</td>
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</tr>
</tbody>
</table>

Conclusion: In this cohort of clinical stage II-III BC patients, AA women were over 75% more likely to have triple negative BC than non-AA patients. Pathologic reponse to chemotherapy did not differ
between AA and non-AA patients. Examining the entire cohort, AA women had worse DFS and OS, however there was no difference among ER-negative subtypes. The difference in DFS and OS was driven by worse outcome among women with ER or PR+ tumors (regardless of HER2). It is possible that this racial disparity in outcome among hormone receptor-positive BC patients reflects unknown biologic differences by race, or differences in access to or receipt of endocrine therapy.

**P2-14-02**

NCIC CTG MA.27: Clinical Tolerability and Overall Survival of Racial and Ethnic Minority Women on Aromatase Inhibitor Therapy.

Moy B, Shepherd LE, Chapman J-AW, Le Maitre A, Gelmon KA, Elliott C, Ingle JN, Goss PE. Massachusetts General Hospital, Boston, MA; National Cancer Institute of Canada Clinical Trials Group, Kingston, ON, Canada; British Columbia Cancer Agency, Vancouver, BC, Canada; Mayo Clinic, Rochester, MN

**Background:** Aromatase inhibitors (AIs) are standard adjuvant therapy for postmenopausal women with hormone receptor-positive breast cancer. We previously reported that racial/ethnic minority women receiving an AI in NCIC CTG MA.17 experienced fewer hot flashes, fatigue, and arthralgias than Caucasians (Moy B et al. Ann Oncol 2006;17:1637). Here we examined whether race affected clinical outcomes in the MA.27 AI trial comparing exemestane (E) with anastrozole (A). Methods: Fisher’s exact test was used to compare observed side effects (0 vs grades 1-5) between minority and Caucasian women. Adverse events (AE), including menopausal symptoms, were assessed according to the Common Terminology Criteria of the National Cancer Institute (version 3.0). ITT univariate test of race effects on overall survival (OS) was determined with a stratified log-rank test, while multivariate testing was with stratified Cox regression. Results: Among 7312 for whom race was known, distribution was: Caucasian (n=6939; 95%); black (n=235; 3%); Asian/Native Hawaiian/Pacific Islander (n=93); 1%; American Indian (n=39; 1%); and mixed race (n=6). Among women treated with E, minorities reported a significantly lower incidence of hot flashes (45% vs. 56%; p=0.003) and fatigue (34% vs. 46%; p=0.001) compared to Caucasians. Similarly with A, minority women reported significantly fewer hot flashes (47% vs. 58%; p=0.002) and lower cholesterol (12% vs. 18%; p=0.01); however, they reported more headaches than Caucasians (16% vs. 10%; p=0.01). Caucasian women were more likely to discontinue therapy due to AE or side effects (32% vs. 24%). There was a significant OS interaction (p=0.02) between race and treatment with minority women on E having fewer deaths than those on A. Caucasian women had HR of E vs A of 0.98 (95% CI 0.81-1.20, p=0.85, while minorities had HR of E to A of 0.72 (95% CI 0.33-1.58, p=0.41. Conclusions: Minority women tolerated AIs better and were also more compliant than Caucasians, supporting our previous findings. A significant interaction between race and type of AI therapy for OS was seen, favoring exemestane in minority women. Replication of these findings in larger cohorts is necessary. Since race/ethnicity may serve as a surrogate for genetic differences, our findings suggest pharmacogenomic differences which require further research.

**P2-14-03**

A Comparison of Biologic Differences in Tumors in a Matched Cohort of Hispanic and Caucasian Women with Early-Stage Breast Cancer Using the 21-Gene Recurrence Score Assay.

Lim EA, Hershman DL, Greenlee H, Crew KD, Mauer MA, Hibshoosh H, Kalinsky K. Columbia University Medical Center, New York, NY; Mailman School of Public Health, New York, NY

**Background:** Several studies demonstrate that Hispanic women have a higher mortality rate and lower incidence of breast cancer (BC) as compared to Caucasian women. This survival pattern has also been observed in Hispanic women with untreated, early-stage BC. The Hispanic population should be considered a heterogeneous group, however, given the various racial and national backgrounds that comprise this entity. As African American (AA) similarly demonstrate a worse survival as compared to Caucasian women, a retrospective analysis of 27 tumor from AA patients (pts) showed a significantly higher expression of the 5 proliferation genes in the 21-gene recurrence score (RS) assay (Oncotype Dx) as compared to other races, with no significant difference observed in the 21-gene RS. The primary aim of this analysis is to investigate biologic differences between Hispanic women in a primarily Dominican Republic population, as compared to Caucasian women, as determined by indices in the 21-gene RS assay.

Methods: We collected data from women with early-stage breast cancer who underwent RS assay testing between 2005 and 2011. Pt charts were reviewed for ethnicity (Hispanic or other), country of origin, RS, 10-year risk of distant recurrence, and breast tumor ER/PR/HER2 expression by Oncotype Dx. Hispanic pts were matched to Caucasians in a 1:2 fashion based on age (+/- 10 years), tumor stage, and presence of lymph node metastases. Prognostically important clinicopathologic features were collected, including lymphovascular invasion (LVI) and grade. Descriptive statistics were computed. Two Sample t-testing was used to evaluate if RS was equal across by ethnicity groups.

Results: Of 214 pts who underwent RS testing, 30 (13.5%) were Hispanic: 18 from the Dominican Republic, 5 from Puerto Rico, and 1 from various Central and South American populations. The 30 Hispanic women were matched to 57 Caucasians: total population 87 pts. The mean RS for Caucasian women was 18.3 (range: 0-54) and for Caucasians: 15.5 (range: 1-38). By two Sample t test, no statistically different differences were observed between Hispanic and Caucasian women in regards to the RS (p= 0.2828) or 10-year distant recurrence score after 5 years of anti-estrogen therapy (p=0.4218). No differences were observed in median ER expression (9.4% vs. 10.1%; Hispanic vs. Caucasian), PR (7.2% vs. 7.7%), or HER2 (9.2% vs. 9.0%). LVI was numerically more frequently identified in Hispanic pts [7/30 (23.3%) vs. 8/57 (14.0%)], as were grade III tumors [7/30 (23.3%) and 4/57 (7%)].

Conclusions: Similar to the findings with AA pts, there was no significant difference in RS between Hispanic and matched Caucasian women with early-stage BCs. A numerical trend to a higher RS was seen in this Hispanic population of primarily pts from the Dominican Republic. We will evaluate for differences in the 5 genes involved in proliferation (CCNB1, MKI17, MYBL2, BIRC5, AURKA). Also, further analyses will be conducted with additional pts to determine if the numeric differences in RS, LVI, and grade are observed in a larger cohort.
P2-14-04
The Influence of Demographic, Psychosocial and Emotional Barriers to Screening for Colorectal and Ovarian Cancer among Latina Breast Cancer Survivors.
Ramirez AG, Holden AE, San Miguel SL, Gallion KJ. UT Health Science Center at San Antonio, San Antonio, TX

Background Ten percent of all new cancers are diagnosed in cancer survivors and second cancers are the sixth leading cause of cancer deaths. Breast cancer survivorship brings to the fore concern that survivors obtain thorough preventive health screening services. In two previous studies, we observed that Latina women are unable to comply with recommendations for breast cancer care due in part to psychosocial barriers including ability to understand physicians and fear of recommendations. Affective influences, particularly depression may also contribute to noncompliance via its inhibitory effect on vigilance. Here we test a hypothesis that similar barriers in addition to depression level restrict breast cancer survivor screening for colorectal and ovarian cancer.

Methods We conducted a cross-sectional study of 117 Latina breast cancer survivors using self-report data. Proportions of respondents compliant and noncompliant with recommended screening protocols were compared. Reasons were coded “yes”-“no”; depression was measured with the Center for Epidemiologic Studies (CESD) instrument dichotomized at the usual cutoff of 16 points or higher signifying depression. Cancer screening compliance was determined by published NCI guidelines. Associations were determined via chi-squared analysis. Multivariate analysis was conducted using logistic regression.

Results Only 5 (4.2%) of participants were screened for both cancers, 43 (36.8%) for either cancer, and 69 (59.0%) were screened for neither cancer. Reported barriers to cancer care were generally high with respect to screening for other cancers, but only “cannot understand English” (66% v 53%, p=0.05) and “care is too expensive” (84% v 71%, p=0.05) were associated with lack of ovarian cancer screening and none were associated significantly with lack of colorectal screening. Thirty-two percent of the sample met CESD criteria for depression, nearly twice the general population rate; it is associated with ovarian but not colorectal screening noncompliance. Factors including unemployment (74% v 57%, p=0.03) and no familial history of cancer (75% v 53%, p=0.02) are related to non-compliance with ovarian screening, but only unmarried status is related to colorectal screening (54% v 20%, p<0.01). Separate multiple logistic regression analyses confirmed the independent significant association of these factors with ovarian and colorectal screening compliance, indicating good model fit and significant proportions of variance explained by the models.

Discussion There are significant impediments to cancer screening among Latina breast cancer survivors. They derive from multiple domains (demographic factors, psychosocial barriers, and affective states). For this reason they require further research to clearly identify them. Moreover we must develop an equally broad-based preventive strategy that addresses each of these domains to promote vigilance and increase healthy behaviors among Latina women.

Acknowledgement This research was possible by grants from the San Antonio Cancer Institute, San Antonio, Texas (P30-CA54174), the Susan G. Komen Breast Cancer Foundation (POP 2000 704), and the National Cancer Institute, Redes En Acción (U01-CA86117).

P2-14-05
Racial Differences in the Use of Adjuvant Chemotherapy for Breast Cancer in a Large Urban Integrated Health System.
Simon MS, Lamartine L, Krajenta R, Boza J, Ruterbusch J, Kunz S, Schwartz K. Karmanos Cancer Institute at Wayne State University, Detroit, MI; Henry Ford Health System, Detroit, MI; Wayne State University, Detroit, MI

Background: Despite improvements in breast cancer treatment, there continues to be a gap in survival between African American (AA) and White women with breast cancer, which may be due at least in part to racial differences in the patterns of care. In order to better understand breast cancer survival disparities, we evaluated racial differences in the receipt of adjuvant chemotherapy among women treated at a large integrated health care system in Southeastern Michigan. Materials and Methods: The study population included 2,234 women (33 % AA) with stage I through III breast cancer treated at the Henry Ford Health System (HFHS) from 1996 through 2005. Linked datasets from the HFHS, the Metropolitan Detroit Cancer Surveillance System (MDCSS) and the U.S. Census Bureau were used to obtain socio-demographic and clinical information. Co-morbidity was assessed by the Charlson co-morbidity index (CCI), and economic deprivation was categorized using a neighborhood deprivation index (DI). Results: AA women were significantly more likely than Whites to have larger tumors (40% vs. 31% > 2 cm), as well as more aggressive tumors (29% vs 19% estrogen and progesterone negative). AA women were also significantly more likely than Whites to have other co-morbid conditions (15 % vs. 8% with a CCI of 2+) and to reside in a more economically deprived area (45% vs. 5% reside in the most deprived area). While AA women were more likely to receive adjuvant chemotherapy (47% vs. 43%, p=0.0278), they were also more likely to have a delay in initiation of treatment (57% vs 45% delayed beyond 60 days from diagnosis, p=0.0004). After multivariable adjustment for age, DI, CCI, insurance and surgery, there were no racial differences in treatment delay (Odds Ratio 1.20, 95% confidence interval, 0.82-1.77). Discussion: In a large urban integrated health care system, racial differences in the delay in receipt of adjuvant chemotherapy for breast cancer can be explained by clinical and socioeconomic characteristics.

P2-14-06
Loch MM, Ross AA, Rosenberg C, Blanchard RA. Boston University Medical Center, Boston, MA; Boston University, Boston, MA

Background: There is limited literature regarding Haitian women and breast cancer. What exists is based on screening with no literature on clinical or pathologic subtypes of breast cancer. We examined patient and tumor characteristics in Haitian born women with invasive breast cancer in our ethnically diverse tertiary care hospital.

Methods: We expanded a pre-existing database to include all women diagnosed with breast cancer from 1998-2011. From the electronic medical record we documented age, body mass index (BMI), place of diagnosis, with mean age 55.1 vs. 59.4 overall p=0.04. Mean age W: 60 days from diagnosis, p=0.0004). After multivariable adjustment for age, DI, CCI, insurance and surgery, there were no racial differences in treatment delay (Odds Ratio 1.20, 95% confidence interval, 0.82-1.77). Discussion: In a large urban integrated health care system, racial differences in the delay in receipt of adjuvant chemotherapy for breast cancer can be explained by clinical and socioeconomic characteristics.
The frequency of HER2-overexpressing breast cancer did not differ among Chinese, Malay, and Indian pts. Indian pts were more likely to have TNBC compared with Chinese (p=0.005) and 50% among Indians (p=0.11, ns). Malay and Indian pts had higher BMI compared to Chinese pts (26.4 and 26.2 vs 22.8, p<0.001 and <0.001 respectively). Malay pts also had lower 5-yr DFS at 59% and 5-yr OS at 76% compared with 76% (p=0.001) and 84% (p=0.04) in Chinese pts. Survival analyses on Indian pts were limited by the small numbers. Full multivariate analyses will be tabulated.

Conclusions: There are significant differences in the frequency of various subtypes of breast cancer among the Chinese, Malays and Indians in Singapore. The disparities in survival outcomes between the Chinese and the Malays may be related to more aggressive tumor biology, more advanced stage at diagnosis and host factors such as higher BMI. The full impact of ethnicity on breast carcinogenesis is still unknown but is increasingly relevant with the development of therapies that target specific molecular pathways.

P2-14-08
Hormone Receptor Status and Breast Cancer Survival among Hispanic and Non-Hispanic White Women over 10 Years of Follow-Up.

Introduction: The joint presence or absence of expression of estrogen receptor (ER+/−), progesterone receptor (PR+/−), and Her2neu (Her2+/−) is now widely used to define the hormone receptor (HR) phenotype of breast tumors. HR phenotype influences breast cancer etiology, response to treatment and survival, which may vary by ethnic group. The triple negative phenotype (TNT: ER-/PR-/Her2-) is associated with the poorest survival and is more common in African American than white women. Few studies have assessed how HR status differs by Hispanic (H) versus non-Hispanic white (NHW) ethnicity in relation to survival.

Methods: We analyzed data from the New Mexico site of the ‘Health, Eating, Activity and Lifestyle’ (HEAL) study for breast cancer mortality over 10 years of follow-up by HR phenotype and ethnicity for 126 H and 408 NHW women with invasive (I-IIIA), first primary breast cancer. Kaplan Meier curves were used to describe differences in breast cancer survival by HR and ethnicity, adjusting for age at diagnosis, stage, and diagnosis of another cancer (yes/no).

Results: Kaplan Meier (KM) analysis indicated that the TNT phenotype was associated with poorer survival, particularly during the first 5 years, although the difference was not statistically significant after 10 years of follow-up (p=0.36). The prevalence of TNT was higher in H (10.3%) than NHW (6.6%) women, and H also tended to poorer survival than NHW women (p=0.17). In multivariate-adjusted models, women with a TNT phenotype had a 26% increased risk of death (HR=1.26 95% CI 0.62-2.6). In NHW women, the risk was 12% (HR=1.12 95% CI 0.43-2.9). Among H women, those with a TNT phenotype had a 17% increased risk of death (HR=1.17 95% CI 0.35-3.8) while those with ER-/PR-/Her2+ had a 46% increase (HR=1.46 95% CI 0.40-5.3).
Discussion: Although this analysis is limited by small numbers, the results suggest that Hispanic women may have worse 10-year survival rates than non-Hispanic women and that women with TNT have a higher risk of death regardless of ethnicity. These differences in survival could be due to a higher rate of recurrence or issues related to treatment. This study suggests that there is an ethnic disparity in survival by HR subtypes that should be examined in a larger population based study.

P2-14-09
Comparison between Spanish and Peruvian Patients with Early Breast Cancer.
Castaneda CA, Gomez HL, Vallejos C, Cortes-Funes H, Castellano D, Andrés E, Cruz W, Ciruelos EM. Instituto Nacional de Enfermedades Neoplásicas, Lima, Peru; Hospital Universitario 12 de Octubre, Madrid, Spain

Purpose: Breast Cancer (BC) is a genetic, heterogeneous disease and has a remarkable variability according to racial factors. Hypothetical explanations for these disparities include differences in tumor biology. The present study was designed to compare clinical and pathological features between Peruvian Latinas and Spanish women with BC; interest of this analysis increases if we take into account the relationship among historic ancestries of both ethnic groups (Incas emporium and Spanish conquers).

Methods: Information was retrospectively reviewed from patients files and pathologic reports from Instituto Nacional de Enfermedades Neoplásicas (INEN) in Lima- Peru, and Hospital Universitario 12 de Octubre in Madrid- Spain. In order to produce comparative information and avoid subjective clinical measurements we selected only non-metastatic and non-bilateral invasive BC cases that underwent surgery as initial therapy. BC cases were classified as molecular subtypes: Luminal A [RE+ and/or RP+, HER2-], Lum B [RE+ and/or RP+, HER2+], triple negative (TN) [RE-, RP-, HER2-] and HER2 [RE-, RP-, HER2+]. Variables were compared with the X2 test and survival curves were evaluated with Kaplan-Meier method.

Results: The study included 3765 BC cases. The Spanish cohort involved 1539 (40.9%) women consecutively diagnosed between 1997 and 2007 (median follow-up of 7.9 years). The Peruvian cohort involved 2226 (59.1%) women consecutively diagnosed between 2000 and 2006 (median follow-up of 6.3 years).

In terms of pathological features, grade 1 tumors were more frequent in Spanish (16.2%) than Peruvian women (9.6%) (p<0.001). Higher rates of lobular histology were also found in Spanish (16.2%) than Peruvian (6.0%) women (p<0.001). Spanish cases presented at earlier stages when evaluated by lymph node status (N0 in 58.9% vs 47.1%) (p<0.001) or by tumor size (T1 in 37.9% vs 17-2%). Conservative surgery were more frequent among Spanish cases (50.6% vs 16.8%) (p<0.001). TN molecular subtype were more frequent among Peruvian cases (22.5% vs 12.4%) (p<0.001).

Brain (10.4% vs5.3%), and skin and subcutaneous (7.1% vs 2.4%) metastases were more frequently found in Peruvian patients. On the other hand, contralateral breast cancer was more frequent among Spanish patients (12.2% vs 2.8%). And when evaluated by molecular subtype, bone metastases in TN were more frequent among Spanish (25.4%) than Peruvian (18.5%) cases.

Disease-free survival rates at 7 years were similar between Spanish and Peruvian patients (80.3% vs 79.6%, p=0.197). However, overall survival at 7 years was better in Spanish women (90.4% vs 82.6%, p<0.001).

Conclusion: Epidemiologic differences in terms of histological features, clinical stage at diagnosis, molecular subtypes distribution, recurrence patterns and prognosis were found among Spanish and Peruvian BC patients in this retrospective analysis.

P2-14-10
Are There Variations in Invasive Tumour Characteristics between Different Ethnic Groups?
Hariri B, Lo M, Gandomiwillja T, Lewis J, Hogben K. Imperial College Healthcare NHS Trust, Charing Cross Hospital, London, United Kingdom

Introduction: Studies have shown that ethnic minorities with breast cancer are more likely to present at a younger age and with more aggressive tumours. The aim of this study was to examine the variation in tumour characteristics and surgical outcomes in different ethnic groups in our breast unit over a 12-month period.

Methods: A retrospective study was performed on all patients with a new diagnosis of breast cancer, either screen-detected or symptomatic, between January to December 2009. Using our Trust databases, data on patient demographics, tumour characteristics, and ethnicity were collected. Where ethnicity was not documented, patients were contacted by telephone to complete the database. Those unable to be contacted were excluded from the study. Other exclusion criteria include those with recurrent ipsilateral breast cancer.

Results: A total of 270 patients (265 females) were included in our study, of which 235 (87%) had invasive cancers (ductal carcinoma, lobular carcinoma, tubular carcinoma, mucinous carcinoma, cribriform carcinoma, papillary carcinoma, sarcoma) and the remaining 35 patients (13%) had non-invasive cancers (ductal carcinoma in-situ, lobular carcinoma in-situ, papilloma, columnar cell hyperplasia). We were unable to contact 23 (8.5%) patients to ascertain their ethnicity, and therefore these have been excluded from our results.

Discussion: Although this analysis is limited by small numbers, the results suggest that Hispanic women may have worse 10-year survival rates than non-Hispanic women and that women with TNT have a higher risk of death regardless of ethnicity. These differences in survival could be due to a higher rate of recurrence or issues related to treatment. This study suggests that there is an ethnic disparity in survival by HR subtypes that should be examined in a larger population based study.

Table 1: Recurrence site of breast cancer in Spanish and Peruvian Triple-Negative Breast Cancer

<table>
<thead>
<tr>
<th>Recurrence location</th>
<th>Triple-Negative</th>
<th>Locoregional</th>
<th>Lung and pleura</th>
<th>Bone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hospital 12 de Octubre</td>
<td>INEN</td>
<td>Madrid</td>
<td>Madrid</td>
<td>Madrid</td>
</tr>
<tr>
<td>Contralateral</td>
<td>5 (7%)</td>
<td>54 (35.3%)</td>
<td>70 (29.6%)</td>
<td>51 (25.4%)</td>
</tr>
<tr>
<td>Locoregional</td>
<td>5 (7%)</td>
<td>54 (35.3%)</td>
<td>70 (29.6%)</td>
<td>51 (25.4%)</td>
</tr>
<tr>
<td>Local</td>
<td>5 (7%)</td>
<td>54 (35.3%)</td>
<td>70 (29.6%)</td>
<td>51 (25.4%)</td>
</tr>
<tr>
<td>Multifocal</td>
<td>5 (7%)</td>
<td>54 (35.3%)</td>
<td>70 (29.6%)</td>
<td>51 (25.4%)</td>
</tr>
<tr>
<td>Diffuse</td>
<td>5 (7%)</td>
<td>54 (35.3%)</td>
<td>70 (29.6%)</td>
<td>51 (25.4%)</td>
</tr>
<tr>
<td>Neural</td>
<td>5 (7%)</td>
<td>54 (35.3%)</td>
<td>70 (29.6%)</td>
<td>51 (25.4%)</td>
</tr>
</tbody>
</table>

Table 1: Tumour characteristics and surgical intervention in patients with invasive breast cancer in 2009

<table>
<thead>
<tr>
<th>Ethnicity (n)</th>
<th>White</th>
<th>Black</th>
<th>Asian</th>
<th>Chinese</th>
<th>Mixed</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>96</td>
<td>12</td>
<td>14</td>
<td>1</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>Mean age (years)</td>
<td>55</td>
<td>62</td>
<td>54</td>
<td>59</td>
<td>59</td>
<td>67</td>
</tr>
<tr>
<td>Age range (years)</td>
<td>20-59</td>
<td>20-59</td>
<td>20-59</td>
<td>20-59</td>
<td>20-59</td>
<td>20-59</td>
</tr>
<tr>
<td>Size of tumour</td>
<td>&lt;15mm</td>
<td>94</td>
<td>93</td>
<td>92</td>
<td>92</td>
<td>92</td>
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<tr>
<td>Mm</td>
<td>15-45</td>
<td>76</td>
<td>76</td>
<td>76</td>
<td>76</td>
<td>76</td>
</tr>
<tr>
<td>Multifocal</td>
<td>14</td>
<td>14</td>
<td>14</td>
<td>14</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>Grade</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Receptor status</td>
<td>ER positive</td>
<td>141</td>
<td>141</td>
<td>141</td>
<td>141</td>
<td>141</td>
</tr>
<tr>
<td>Receptor status</td>
<td>TR positive</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Surgical intervention</td>
<td>Mastectomy</td>
<td>39</td>
<td>39</td>
<td>39</td>
<td>39</td>
<td>39</td>
</tr>
</tbody>
</table>

Conclusion: We found that the age of breast cancer presentation was lowest in Black and Asian patients (mean age 52 years and 54 years, respectively), compared to White (mean age 59 years). Furthermore, compared to white patients, tumours in Asian patients were larger (62.5% vs 47.2%), of higher grade (83.3% vs 75.2%), more likely...
to be ER negative (16.7% vs 12.4%), and tended to be lymph node positive (41.7% vs 34.2%). These may explain the higher mastectomy rate in Asian patients (n=12, 50%).

The reason for the younger presentation age in the ethnic Asian group is unclear. However, it is possible that their cancer may have different tumour biology. Other studies have suggested reasons such as birth cohort effect and obesity.

Reflecting the United Kingdom’s population the majority of patients that present to our breast unit are White and the number of Asian patients remains small. An increase in the sample size may strengthen our results.

**P2-15-01**

**Surgical Patterns of Care after Magnetic Resonance Imaging in the Academic Setting in Patients with Operable Breast Cancer Treated with Neoadjuvant Systemic Therapy: A Secondary Analysis of TBCRC 017.**

De Los Santos J, Cantor A, McGuire K, Golshan M, Meric-Bernstam F, Horton J, Nanda R, Amos K, Forero A, Hudis C, Meszoely I, Hwang S, University of Alabama at Birmingham, Birmingham, AL; University of North Carolina Chapel Hill, Chapel Hill, NC; Dana-Farber Cancer Institute, Boston, MA; Duke University Medical Center, Durham, NC; Memorial Sloan Kettering Cancer Center, New York, NY; University of Pittsburgh Medical Center, Pittsburgh, PA; MD Anderson Cancer Center, Houston, TX; Vanderbilt University, Nashville, TN; University of Chicago, Chicago, IL; University of California at San Francisco, San Francisco, CA

Background: Neoadjuvant chemotherapy is increasingly used to downstage locally advanced tumors, test new regimens, and improve chances of breast conservation. In parallel, use of breast MRI for surgical planning and as a potential response biomarker has also increased along with an observed increase in mastectomy rates. This secondary analysis reports surgical patterns of care across 8 NCI comprehensive cancer centers in women receiving both neoadjuvant therapy and breast MRIs.

Methods: 770 women from 8 institutions were retrospectively identified as having received neoadjuvant systemic therapy with MRI done before and after systemic treatment. Univariate and multivariate analyses of covariates associated with surgical management were performed. Within MRI complete response or not, the Jonckheere-Terpstra Test (JTT) was used to test for a trend in mastectomy rate by T stage.

Results: Surgical data was available on 763/770 patients. Table 1 lists MRI response by pretreatment imaging size and final breast surgery. Table 2 lists demographics, tumour characteristics and reasons for mastectomy.

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**Table 1: Type of surgery performed in patients with invasive breast cancer**

<table>
<thead>
<tr>
<th>Years</th>
<th>Lumpectomy</th>
<th>Mastectomy</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000</td>
<td>26 (15)</td>
<td>23 (14)</td>
</tr>
<tr>
<td>2005</td>
<td>22 (16)</td>
<td>21 (15)</td>
</tr>
<tr>
<td>2009</td>
<td>9 (10)</td>
<td>7 (8)</td>
</tr>
</tbody>
</table>

**Table 2: Indications for mastectomy in patients with invasive breast cancer in 2009**

<table>
<thead>
<tr>
<th>Number of patients</th>
<th>Screen-detected (a)</th>
<th>Symptomatic (a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of patients</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean age (years)</td>
<td>59</td>
<td>58</td>
</tr>
<tr>
<td>Age range (years)</td>
<td>31-66</td>
<td>25-86</td>
</tr>
<tr>
<td>Size of invasive cancers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Size of invasive cancers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>Indications for mastectomy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multifocal lesion</td>
<td>5</td>
<td>35</td>
</tr>
<tr>
<td>Large tumour in relation to breast size</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Location of tumour</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient choice</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family history of breast cancer</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Fear of further treatment</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

---

**P2-15-02**

**Are We Performing More Mastectomies on Women Diagnosed with Invasive Breast Cancer?**

Lo MCI, Hariri B, Gandamahirdja T, Lewis J, Hogben K. Imperial College Healthcare NHS Trust, Charing Cross Hospital, London, United Kingdom

**Introduction**

Surgical management of breast cancer has changed over the past several decades; breast-conserving surgery has become widely accepted. Mastectomies, however, are still being performed as part of the management of breast cancer. The aim of this study was to investigate the indications for mastectomy in women with invasive breast cancer within our institution.

**Method**

A systematic review of all new patients with invasive breast cancer treated with surgical intervention at our breast unit in the years 2000, 2005 and 2009 was performed. Using our databases, patient demographics, tumour characteristics and reasons for mastectomy were examined. Screen-detected and symptomatic patients were included in the study. Patients excluded were those with incomplete data and recurrent breast cancer.

**Results**

A total of 584 patients (F:M, 576:8); with cancer types which included invasive ductal carcinoma (n=501, 84%), invasive lobular carcinoma (n=51, 9%), invasive tubular carcinoma (n=27, 5%) and sarcomas (n=5, 1%) were studied.

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**Table 3: Indications for mastectomy in patients with invasive breast cancer in 2009**

<table>
<thead>
<tr>
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<td></td>
</tr>
<tr>
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<td>2</td>
</tr>
<tr>
<td>Fear of further treatment</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

---

**Notes:**

- Fear of further treatment
- Family history of breast cancer
- Tumours with sizes not recorded

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**P2-15-03**

**Secondary Analysis of All Patients With Invasive Breast Cancer Treated With Neoadjuvant Systemic Therapy: A Secondary Analysis of TBCRC 017.**

De Los Santos J, Cantor A, McGuire K, Golshan M, Meric-Bernstam F, Horton J, Nanda R, Amos K, Forero A, Hudis C, Meszoely I, Hwang S, University of Alabama at Birmingham, Birmingham, AL; University of North Carolina Chapel Hill, Chapel Hill, NC; Dana-Farber Cancer Institute, Boston, MA; Duke University Medical Center, Durham, NC; Memorial Sloan Kettering Cancer Center, New York, NY; University of Pittsburgh Medical Center, Pittsburgh, PA; MD Anderson Cancer Center, Houston, TX; Vanderbilt University, Nashville, TN; University of Chicago, Chicago, IL; University of California at San Francisco, San Francisco, CA

Background: Neoadjuvant chemotherapy is increasingly used to downstage locally advanced tumors, test new regimens, and improve chances of breast conservation. In parallel, use of breast MRI for surgical planning and as a potential response biomarker has also increased along with an observed increase in mastectomy rates. This secondary analysis reports surgical patterns of care across 8 NCI comprehensive cancer centers in women receiving both neoadjuvant therapy and breast MRIs.

Methods: 770 women from 8 institutions were retrospectively identified as having received neoadjuvant systemic therapy with MRI done before and after systemic treatment. Univariate and multivariate analyses of covariates associated with surgical management were performed. Within MRI complete response or not, the Jonckheere-Terpstra Test (JTT) was used to test for a trend in mastectomy rate by T stage.

Results: Surgical data was available on 763/770 patients. Table 1 lists MRI response by pretreatment imaging size and final breast surgery. Table 2 lists demographics, tumour characteristics and reasons for mastectomy.

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<tr>
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**Table 2: Indications for mastectomy in patients with invasive breast cancer in 2009**

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<td></td>
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<tr>
<td>Family history of breast cancer</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Fear of further treatment</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>
Conclusion
In our institution, the majority of patients with screen-detected invasive breast cancer fulfilled the criteria to have breast-conserving surgery; mastectomy rate for screen-detected cancers remained stable. However, we found that in the symptomatic patient group, there was an increase in mastectomy rate from 16% (n=170) in 2000 to 52% (n=225) in 2009. Despite this trend, our results were still in keeping with the national standard as illustrated by the National Cancer Intelligence Network, where they noted that in 2006, 52% of symptomatic patients with invasive breast cancer underwent mastectomy in the United Kingdom.

The reason for a higher mastectomy rate in symptomatic patients compared to screen-detected patients in 2009 is multifactorial. We found that a larger proportion of symptomatic patients presented with larger tumours and tumours which were multifocal in nature. Other factors which may have contributed included more accurate estimation of tumour size by imaging (e.g. MRI) and patient choice.

P2-15-03
Sakurai T, Sakurai T, Umemura T, Jinta E, Suzuma T, Yoshimura H, Ohsumi S, Iwase T, Inaji H, Mitzutani M, Nishimura R, Mukai H, Niigata Cancer Center Hospital, Niigata, Japan; National Hospital Organization Shikoku Cancer Center, Matsuyama, Japan; Cancer Institute Hospital Tokyo, Japan; Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka, Japan; National Hospital Organization Osaka National Hospital, Osaka, Japan; Kansai Medical University, School of Medicine, Osaka, Japan; Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka, Japan; National Hospital Organization Osaka National Hospital, Osaka, Japan; Kumamoto City Hospital, Kumamoto, Japan; National Cancer Center Hospital East, Kashiwa, Japan

[Introduction] Mastectomy has been conducted in general for cases in which breast conservation is inapplicable. Since Nipple-sparing Mastectomy (NSM) conserves nipple-areola, it is not only more advantageous in an aspect that nipple-areola reconstruction isn’t required in case of breast reconstruction compared with mastectomy but also significantly meaningful because the sensation of nipple-areola is preserved in about 70% cases by skin flap preparation based on thick flap method we have adopted. On the other hand, NSM is controversial relating to complication of surgery and recurrence as well.

[Objective] We’ve made (1) review of safety in NSM surgical technique, (2) discussion over recurrence and prognosis comparing with those in mastectomy, and (3) consideration of performance in immediate reconstructions.

[Result] (1) Of all cases of NSM, the number of cases in stage 0, 1, 2A, 2B, 3 and 4 was 21, 305, 239, 123, 112 and 6 respectively. During 104 months of follow-up period in average, total of seven complication cases (0.9%) were observed, including two flap necrosis cases and five postoperative bleeding cases, but no nipple necrosis case was recorded. Although nipple-areola recurrence rate (%) in stage 0, 1, 2A, 2B and 3A was 0, 2.9, 4.6, 2.4 and 5.3 respectively, prognosis of nipple-areola recurrence cases was good showing no difference between stages (OS: 60M; 93%, 100M; 84%). Skin flap recurrence rate (%) in stage 0, 1, 2A, 2B and 3 was 0, 3.2, 3.3, 4.0 and 14.2 respectively, and prognosis of skin flap recurrence cases was significantly worse in stage 2B and 3 compared with that in stage 1 and 2A (p < 0.001).

(2) We analyzed local recurrence rate, recurrent free survival and overall survival between 806 of NSM cases and 200 of mastectomy cases which had been performed during the same period, and the result showed no significant difference in those rates between NSM and mastectomy cases.

(3) Infectious complication was observed in one out of 18 immediate reconstruction cases using free dermal fat graft. Among 124 immediate reconstruction cases using implant, complications were observed in five postoperative bleeding.

[Conclusion] NSM was excellent in cosmetic aspect showing equivalent results in local recurrence rate and prognosis compared with those in mastectomy cases. Besides, patient’s satisfaction was also higher in those who had had NSM alone without reconstruction. Our long term follow-up data shows that NSM may be considered as an alternative option for mastectomy in cases in which breast-conserving surgery is inapplicable in breast cancer patients.

P2-15-04
Clinical Significance of Resection with Curative Intent for Isolated Pulmonary Metastases from Breast Cancer. Multi-Institutional Study in Japan.
Sato N, Ohsumi S, Iwase T, Inaji H, Mitzutani M, Nishimura R, Mukai H, Niigata Cancer Center Hospital, Niigata, Japan; National Hospital Organization Shikoku Cancer Center, Matsuyama, Japan; Cancer Institute Hospital Tokyo, Japan; Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka, Japan; National Hospital Organization Osaka National Hospital, Osaka, Japan; Kumamoto City Hospital, Kumamoto, Japan; National Cancer Center Hospital East, Kashiwa, Japan

OBJECTIVE: Although resection of isolated pulmonary metastases is a common treatment in other primaries such as colon cancer, the role of lung metastasectomy of breast cancer is still unclear. The objective of the present study was to investigate the clinical outcome of our operated patients with isolated pulmonary breast cancer metastases and discuss the clinical implication of resection with curative intent for them.

METHODS: We retrospectively analyzed 86 female patients with histologically proven isolated pulmonary metastases from breast cancer who were treated with surgery of curative intent between January 1980 and September 2010 at 7 Japanese hospitals. The mean age of them was 50 years, the median disease free interval (DFI) from the definitive surgery for the primary breast cancer was 4.6 years (range: 0.5 - 20.4 years) and the median follow-up after lung metastasectomy was 4.1 years (range: 0.3 - 30.9 years).

RESULTS: Lung metastasectomy was performed by thoracotomy in 22 patients and by video-assisted thoracoscopic surgical resection in 64 patients. The number of metastatic foci resected was one for 79 patients, two for 6, and unknown for 1. Lymph node resection was performed for 12 patients, was not done for 69, and unknown for 5. Five-year survival rates after lung metastasectomy were 68% in the patients. The main prognostic factor was the DFI. Five-year survival rates for patients with DFI of longer than 2 years (n = 72) and those with DFI of shorter than 2 years (n=14) were 74.5% and 33.8%, respectively (p = 0.0006). Five-year survival rates after lung metastasectomy were 68% in patients without macroscopic residual pulmonary tumor after lung metastasectomy (n = 69) and 34% in those with macroscopic residual tumor (n = 12) (p = 0.023). Five-year disease free survival after lung metastasectomy for patients without macroscopic residual tumor was 51 %. Five-year survival rates were 78% in patient with endocrine hormonal therapy following lung metastasectomy (n = 51) and 53% in those without endocrine hormonal therapy (n = 35) (p = 0.045). No prognostic influence could be demonstrated for age, number of initial axillary node metastases, primary tumor size.

CONCLUSION: The prognosis of the breast cancer patients with isolated pulmonary metastases who underwent metastasectomy with curative intent was good. It was affected by the biological aspects
of the primary tumor, “curativity” of the resection, and systemic treatments after lung resection. It may be expected that some of those patients will be cured by surgery with curative intent.

P2-15-05
Excision of the Primary Tumour in Patients with Metastatic Breast Cancer – Will E2108 Provide the Definitive Answer?

Background: Approximately 10% of new breast cancer patients will present with overt synchronous metastatic disease. Controversy exists about the optimal local management of these patients. While several series suggest that removal of primary tumour is associated with a survival benefit, the retrospective nature of these studies raises considerable methodological challenges. We decided to evaluate the experience at our centre around the impact of surgery in patients with synchronous metastasis.

Method: Case records of all patients seen with primary breast cancer and concurrent distant metastases between 2005 to 2007 were reviewed. Demographic and treatment data was collected. The study endpoints compared both overall survival and symptomatic local progression rates between patients who had breast surgery and those who did not.

RESULTS: 111 patients were identified. Median follow-up 40 months (0.6-71 months). Patients were divided into two groups: those patients who underwent breast surgery (n=48; 29/48 had surgery immediate prior to metastatic diagnosis) and those that did not have surgery(n = 63). The surgical group were less likely to present with T4 tumours (20% vs 36%), N3 nodal disease (8% vs 19%) and visceral metastasis (67% vs 73%) when compared with non-surgical group. Improved overall survival (49 months vs 33 months; p=0.01) and less symptomatic local progression rates (15% vs 43%, p < 0.001) were seen in the surgical group compared to the non-surgical group.

CONCLUSIONS: The optimal local management of patients with metastatic breast cancer is unknown. Despite the surgery group demonstrating an improved overall survival and symptomatic local control, this group had less aggressive disease at presentation. These results confirm the need for prospective randomized studies. E2108, an ongoing Phase III Trial, was designed to assess the effect of breast surgery in metastatic patients responding to first line systemic therapy. If excision of the primary tumour is associated with a survival benefit, then the pre-selected subgroup of patients that have responded to initial systemic therapy is the desired population to put this hypothesis to test.

P2-15-06
Breast Cancer Liver Metastases – Possibilities and Limits of Surgical Treatment.
Narsanska A, Treskova I, Treska V, Skallicky T, Sutnar A. University Hospital and Medical Faculty Pilsen, Charles University Prague, Pilsen, Czech Republic

Background: Breast cancer liver metastases (BCLM) are often considered as a sign of a systemic disease with little hope of therapeutic success. The aim of study was to assess the possibilities and determine the limits of the surgical treatment of BCLM.

Method: 646 patients were operated for malignant and benign lesions of the liver between 1999 and 2010. Liver surgery for BCLM was performed in 21 women of the average age of 48.5 years (33-71). The average time from the primary surgery for breast cancer till BCLM diagnosis was 4.7 years (2 months - 9 years). BCLM were solitary in 17 and in four cases multiple. Patient selection for liver surgery was based on sufficient future remnant liver volume, the absence of systemic non-resectable tumor dissemination and response to chemotherapy. The authors performed six right-sided hepatectomy, four segmentectomies, three left lobectomies, one metastasectomy, six radiofrequency ablations (RFA), one combined procedure - liver resection and RFA. Histological examination revealed ductal carcinoma in fourteen and lobular carcinoma in seven cases. All patients were treated with the curative adjuvant chemotherapy after surgery. The data were statistically evaluated by statistical software Statistica 9.0.

Results: 30 – days mortality rate was 0%. One patient had a complicated hepatectomy with iatrogenic bile duct injury. According to statistical analysis the probability of patients survival twelve, resp. thirty months after surgery was 100, resp. 66.7% and the probability of the tumor relapse anywhere in the body was at the same time intervals 0, resp. 71. 5%.

Conclusion: Liver surgery combined with the adjuvant chemotherapy are a therapeutic methods of choice for highly selected patients with metastases limited to the liver and objective response to neoadjuvant chemotherapy.

The study was supported by the research projects IGA MZ NS 9727 and IGA MZ NS 102 40.

P2-15-07
The Need for Additional Surgeries to Adequately Excise Early Breast Cancers May Have a Negative Impact on Local Recurrence.
Warburton R, Alriyees L, Wang DY, Wong SL, Leong W. University Health Network, University of Toronto, Toronto, ON, Canada

Background: Breast conservation surgery (BCS) is considered the standard of care for the treatment of early stage breast cancer (ESBC, AJCC stage 1 and 2). 20 to 50% of patients will require further surgery for positive or close margins. There is recent data suggesting that re-operation can have a negative impact on local and distant recurrences. Our aim is to examine the effects of multiple surgeries to obtain adequate margins on breast cancer recurrence, metastatic disease and survival.

Methods: We reviewed a prospectively maintained breast cancer database at the University Health Network and included all women who had BCS for their first diagnosis of ESBC between January 2004 and December 2007. Patients with neoadjuvant chemotherapy were excluded. We collected patient demographics, surgical pathology, adjuvant therapy and follow up outcome data, which included local recurrence, distant recurrence, cancer-specific survival and overall survival. Clinical and pathologic features were compared using chi-square analyses. Patients who had one lumpectomy were compared to those who had multiple surgeries by using Kaplan-Meier survival curves and log rank test.

Results: Of a total of 744 patients (8 patients had bilateral cancer) 577 (77.6%) had one lumpectomy only (Group 1). 167 (22.4%) patients required further surgery (group 2 = LR+LM+LRM); 83 (11.1%) had a re-excision (LR), 69 (9.3%) had mastectomy (LM) and 15 (2%) had a re-excision followed by a mastectomy (LRM). Thus, a total of 85 (11.4%) patients had mastectomy to achieve adequate margins. All clinicopathologic factors and adjuvant systemic treatments were similar between the two groups except for age and use of adjuvant radiotherapy, which was related to that fact that many of those in group 2 had mastectomy. We observed a difference in disease-free survival favoring patients with lumpectomy only (group 1- 3.4% vs. group 2 - 8.1%, p= 0.01) but there was no difference in distant
metastasis (4.5% vs. 5.6%, p=0.56), cancer-specific survival (97.6% vs. 95.6%, p=0.20) and overall survival (6.9% vs 6.3%, p= 0.76) at a median follow up of 4.54 years.

Conclusion: Despite having similar stage, grade, receptor status and adjuvant systemic therapies, having multiple surgeries for primary breast cancer appeared to be associated with decreased disease-free survival but had no difference in rates of distant metastatic disease, cancer-specific survival and overall survival.

**P2-15-08**

**New Trends for Surgical Treatment of Large Breast Tumors.**

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**Introduction:** The locally advanced breast cancer is still common in developing countries. The neoadjuvant chemotherapy (NC) allows converting large tumor into operable one. Oncoplastic surgery (OS) adds new techniques to breast conservative treatment (BCT). The association between NC and OS provides the resection of large amount of breast tissue, including skin, with satisfactory aesthetic results.

**Objective:** Evaluate the possibilities to indicate oncoplastic techniques to determine BCT after NC for large breast tumors.

**Material and Methods:** A prospective clinical trial (www.clinicaltrials.gov; NCT00820690) study with women breast cancer patients, clinical stage III, no extent skin involvement, submitted to NC, based on four cycles of doxorubicin 60mg/m2 + cyclophosphamide 600 mg/m2 and 4 cycles of paclitaxel 175mg/m2. Preoperative tattoo was realized for all patients to determine the clinical dimensions of the tumor. Tumor size was controlled by physical and radiological exams. The oncoplastic surgeries were offered in accordance to clinical-imaging previous to NC, final response to chemotherapy, tumor localization, tumor size, breast size, oncologic security, patient comorbidities and experience of the surgeon. The skin-sparing mastectomy with immediate breast reconstruction was also considered an oncoplastic technique. The surgical planning has involved the whole area previously marked by the tattoo regardless of tumor response. A detailed analysis of the pathological specimen was provided to confirm the oncologic safety of the treatment. All patients were followed by radiotherapy after surgery.

**Results:** 50 patients were enrolled. PE tumor median measurement was 6.5 cm (3.0 to 14.0 cm) and pathologic median tumor was 4.0 cm (0 to 14.5cm). Pathologic response was rated as stable disease, progressive disease, partial response and complete response in 18%, 10%, 68% and 4% of the cases, respectively. Pathologic responses were diverse and included tumor micro-fractionation and macro-fractionation. PE tumor size before chemotherapy correlated best with MRI (rcc 0.588, p <0.001), as well as pathologic tumor size measured after chemotherapy correlated best with MRI (rcc 0.738, p <0.001). The surgical approach allowed BCT in 34% (17) of patients. Skin involvement was presented in 38% of pathologic specimen regardless previous clinical exam noticed.

**Conclusion:** Oncoplastic techniques increase the rates of BCT for selected patients in spite of large locally advanced tumors. The surgical planning is fundamental to this approach. This study recommends the full resection of the tumoral area previous to NC, including marked skin, regardless the response to neoadjuvant treatment. Follow-up of these patients will be important for additional information in the future.

**P2-15-09**

**Prospective Randomized Comparison of Conventional Instruments and the Harmonic Focus® Device in Breast-Conserving Therapy for Primary Breast Cancer.**

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**Background:** In recent years, surgeons have utilized Harmonic instruments to perform breast cancer resection. Retrospective and prospective studies have demonstrated that the use of this surgical device for mastectomy and axillary dissection can reduce perioperative blood loss, seroma formation, and duration and total amount of drainage. No study has analyzed the feasibility of Harmonic instruments in breast-conserving surgery. We conducted a prospective, randomized clinical trial comparing Harmonic instrument and conventional surgery in the performance of breast conserving surgery and axillary procedures to determine differences in surgical procedures, postoperative outcome, and complications.

**Methods:** One hundred and forty seven patients with operable breast cancer who underwent breast-conserving surgery at a single institution between December 2009 and March 2011 were included in the analysis. Surgery was performed in 73 patients with the Harmonic Focus® device and in 74 with scissors and electrocautery. Charts were reviewed for patient demographics, histopathologic reports, surgical procedures, length of stay, volume and duration of postoperative drainage, complications such as seroma and hematoma formation, and postoperative pain.

**Results:** We found a statistically significant difference in size of resected breast tissue (p=0.001), postoperative serum hemoglobin (p=0.001), volume (p<0.001) and duration (p=0.001) of breast drainage, volume (p<0.001) and duration (p=0.001) of axillary drainage, intramammary (p=0.04) and axillary (p=0.006) seroma formation, subjective (p=0.012) and objective (p=0.001) postoperative pain, and length of hospital stay (p=0.002) in favor of the Harmonic surgical device.

**Discussion:** This is the only study comparing the Harmonic instrument with traditional surgery in both breast conserving surgery and axillary procedures. From this study, we conclude that despite higher costs, the Harmonic device is safe to use and provides key benefits in intraoperative technique, postoperative outcome, and rate of complications in breast cancer surgery.

**P2-15-10**

**Do Patients with Incurable Advanced Breast Tumor and Distant Metastasis Derive Any Benefit from Primary Tumor Resection?**

Takahashi M, Shibasaki S, Jotoku H, Watanabe K-I. Hokkaido Cancer Center, Sapporo, Hokkaido, Japan

**Background:** Metastatic breast cancer (MBC) is considered incurable. Surgical removal of primary tumors has not been established as a standard treatment for MBC because it is generally accepted that local therapy provides no survival advantage once metastases have occurred. Additionally, tumor excision may further stimulate metastasis. Therefore, primary tumor resection in patients with MBC is usually only applied as a palliative treatment for symptomatic wound complications such as bleeding, ulcer formation, unpleasant smell, and purulent discharge. In recent years, however, some retrospective studies have reported advantages of debulking surgery in terms of improving patient outcome. We retrospectively analyzed the surgical benefits and prognostic factors for patients with MBC who were treated at our center.

**Methods:** We retrospectively reviewed individual medical records from the Hokkaido Cancer Center. In this study, incurable advanced
breast cancer is defined as the presence of a tumor, larger than 5 cm, and shows either (invasion to chest wall or skin or is an inflammatory carcinoma) and at least one metastatic site, including distant lymph nodes, bone, or visceral organs (lung, pleura, mediastinum, liver, and brain) at diagnosis. Between January 2000 and June 2010, 92 women were diagnosed with incurable advanced breast cancer at our institute. The effect of surgical treatment on survival was evaluated. Patient demographics and tumor characteristics were also investigated.

**Results:** Thirty-six patients had surgery for resection of primary tumors. There were no substantive differences between individuals, or between tumor characteristics, for patients who underwent surgery versus patients who did not. The median survival time for surgically treated patients was 25.0 months versus 24.8 months for patients who did not undergo surgical resection (P=0.352). Only three patients relapsed within three months of surgery. For the remaining majority of patients, primary tumor resection gave some relief from the often severe symptoms that come from harboring a large tumor for an extended time. In univariate and subsequent multivariate analyses of predictive indicators, a diagnosis of triple-negative breast cancer and/or metastasis to more than three sites was significantly associated with a severe prognosis.

**Conclusion:** Primary tumor resection failed to prolong overall survival times in patients with incurable advanced breast cancer that was greater than 5 cm. However, surgery did improve the quality of life in patients who were expected to have a relatively long prognosis.

**P2-15-11**

Outcomes of Corrective Procedure with Vicryl Mesh as an Oncoplastic Surgery of the Breast.

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Background: Both cosmetic and oncologic outcomes are becoming more important for breast cancer patients. Breast-conserving surgery (BCS) has an acceptable cosmetic result compared with mastectomy, but BCS is also associated with cosmetic failure. In this study, we evaluated the cosmetic outcome of reconstructive surgery with the absorbable implant vicryl mesh after BCS compared with the outcome of BCS alone.

Methods: From May 2007 to December 2009, 101 patients were involved in this study at Hallym University Sacred Heart Hospital. We used absorbable implants made of folding vicryl mesh and wrapped them with Interceed. In 79 cases, BCS with vicryl mesh implantation was performed; in the other 56 cases, only BCS was performed. Patient satisfaction with cosmetic outcomes was evaluated. We also analyzed other factors that affected cosmetic outcomes, including patient age, body mass indices, tumor locations, and removed breast tissue.

Results: In the vicryl mesh implantation group, 65 of 79 patients (82.3%) were satisfied; in the BCS-only group, 24 of 56 patients (43%) were satisfied (P < 0.05). In the vicryl mesh implantation group, patient ages, body mass indices, and removed breast tissue did not affect cosmetic outcomes. When the tumor was located in the upper outer quadrant, the patients were more satisfied (P < 0.05).

Conclusions: Reconstructive procedures with vicryl mesh are simple, safe, and less expensive than other plastic reconstruction techniques. This study suggests that the procedure was superior to BCS alone in cosmetic outcomes. We believe that the procedure could become a favorable technique in oncoplastic surgery.

**P2-15-12**

Management Strategy of Early-Stage Breast Cancer Patients: With or without Axillary Lymph Node Dissection.

Yamamoto D, Tanaka K, Tsubota Y, Yosida H, Kanematsu S. Kansai Medical University, Hirakata, Japan; Ribon-Roze Tanaka Kanji Clinic, Osaka, Japan

Background: The sentinel lymph node (SLN) biopsy has steadily replaced axillary lymph node dissection (ALND) for staging clinically node-negative breast cancer. However ALND remains the standard management of the axilla when a tumor-positive sentinel lymph node (SLN) is identified. The aim of this study was to evaluate the ALND in local, distant recurrence, and overall survival (OS) in breast cancer patients.

Methods: We identified 350 patients with breast cancer (clinically T1/T2N0M0) from the prospective database from five institutions from July 1999 and April 2004. All patient age ranged from 26 to 81 (median 49) years. All patients underwent mastectomy or breast conserving surgery (BCT) combined with either ALND or no ALND.

Criteria of negative ALN metastasis are that minor axis of ALN is under 5 mm on CT images and fat tissue in the hilum of ALN does not disappear on ultra-sound images. In the case that hormone receptor expression is positive, pt was administered for 5 years. In the case of T1c or T2 under 70 years old, neoadjuvant and/or adjuvant anthracycline-based chemotherapies were administered. After surgery, patients without ALND with ALND received tangential irradiation at the region of breast. ALND and no ALND groups were compared for incidence of local, regional, distant recurrence, and overall survival.

Results: Patients with ALND (n=350) were compared with no ALND group (n=126). There were significant differences in primary tumor size, histology, nuclear grade, presence of LVI, ER/PgR/HER2 status between the two groups. Five years OS was 97.9% in ALND group, it was 98.0% in no ALND group (p<0.01). Further there was no significant difference between two groups in local, regional, and distant recurrence.

Conclusions: These results indicate that ALND is omissible for cT1/T2N0M0 breast cancer by a combination of hormone therapy, neoadjuvant/adjuvant chemotherapy, and irradiation.

**P2-15-13**

Oncologic and Cosmetic Outcome in Breast Cancer Patients Who Underwent “Moving Window” Operation.

Ohno Y, Noguchi M, Nakano Y, Noguchi M, Kosaka T. Kanazawa Medical University, Japan

Background: An inappropriate skin incision on the breast reduces the cosmetic benefit of breast-conserving surgery (BCS).

Methods: To improve the cosmetic outcome, we have performed “Moving window” operation in which BCS can be performed via a periareolar incision (periareolar approach) and/or axillary incision (axillary approach) under direct visualization. Axillary lymph node dissection is also performed via an axillary incision.

Results: Periareolar approach was performed in 65 patients and axillary approach in 43 patients. Average operation time was 130 minutes in periareolar approach and 131 minutes in axillary approach. Average blood loss was 37 mL and 50 mL, respectively. Postoperatively, the surgical margin of breast tissue was histologically confirmed to be negative in 107 (99%) of 108 patients, while two patients underwent reoperation because of positive surgical margin. Fifty-two patients (85%) in periareolar approach and 37 patients (86%) in axillary approach had excellent or good cosmetic results.
With a mean follow-up of 36 months, one patient with DCIS developed in-breast recurrence, while 3 patients who had neoadjuvant chemotherapy developed in-breast recurrence. 

Conclusion: The moving window operation can improve a cosmetic outcome of the conserved breast without compromising the oncological safety. Moreover, it can reduce operating time and blood loss when compared with the endoscope-assisted BCS.

**P2-16-01**

A Multi-Centre Prospective Cohort Study Evaluating Health Related Quality of Life after Types of Immediate Latissimus Dorsi (LD) Breast Reconstruction.


**Introduction:** Evidence for the clinical effectiveness of breast reconstruction based on Patient Reported Outcome Measures (PROMS) is lacking. Methodology evaluating PROMS after types of breast reconstruction has been poor with respect to study design, statistics, missing data and absence of prospective documentation of pre-defined complication data in a systematic review of all studies since 1978. Furthermore, there is no reliable data on the effects of associated radiotherapy (RT) in this context. As a prelude to a proposed randomised trial in breast reconstruction, our aim was to conduct a 'robust' cohort study evaluating the effects of either implant-assisted LD (LDI) or tissue only (ALD) LD flap reconstruction in relation to key determinants including clinico-pathological parameters, complications and treatment schedules over a 36 month period.

**Methods:** An MREC approved prospective longitudinal cohort study involving 6 centres commenced in early 2007. Serial PROMS using the EORTC QLQ-C30, BR-23, FACT-B, BIS and HADS, were completed pre-operatively and at 3, 6, 12, 24 and 36 months after surgery. Data up to 12 months were included in this analysis as data were sparse beyond this point; follow-up is ongoing. Demographic and clinical data were compared between the surgical groups. Generalised estimating equations were used to investigate demographic and clinical predictors of HRQL over time.

**Results:** A total of 189 patients (107 – ALD, 82 – LDI) were recruited, with a mean age of 50 years (range 22-70). Baseline questionnaires were completed by 149 (79%) women, with 167 (88%) available surveys (51% response rate). MO and PS had similar overall knowledge scores (MO 59%, PS 56%, p=0.5). 23% of MO knew the correct treatments for breast skin necrosis, and 60% knew the correct treatment for seromas. 32% of PS knew the approximate duration of Her2-directed therapy, and 14% of PS identified the usual adjuvant treatment for patients who have had neoadjuvant chemotherapy. Providers from both specialties who primarily practice in a rural setting scored significantly lower on the knowledge portion of the survey than those practicing in an academic medical center or urban/suburban area (51% rural versus 59%, p=0.03).

The MO and PS agreed on the MO’s degree of responsibility for timely chemotherapy initiation (MO mean 4.6 versus PS mean 4.4 out of 5, p =0.2). However, they disagreed about the PS’s responsibility for timely chemotherapy initiation (MO mean 3.8, PS mean 3.0 out of 5, p=0.01). MO attributed low importance to a history of immediate breast reconstruction when planning chemotherapy treatment (mean 2.5 out of 10). PS placed little importance on the likelihood of post-operative chemotherapy when planning immediate breast reconstruction (mean 3.6 out of 10). Both MO and PS reported more frequent communication with each other about patients with complications (p<0.01 for various subscales). Significant improvements over time were noted for global QoL, role and social functioning, fatigue, pain and breast symptoms (p<0.001 for all).

**Conclusion:** There is increasing evidence of clinical equipoise between types of LD breast reconstruction and despite acknowledged cosmetic disadvantages the overall effects of PMRT on HRQL are minimal. The identification of important variables that may affect HRQL is crucial in all studies evaluating the effects of surgery on PROMS. Their integration into study results is essential for correct interpretation of clinically based assessments. This remains a challenging aspect in cohort studies, and emphasises the need for pragmatism in design of trials in the field.


**P2-16-02**

How Well Do Medical Oncologists and Plastic Surgeons Communicate about Their Patients?

Milucky JL, Deal A, Wu R, McNally RS, Anders C, Lee C. University of North Carolina at Chapel Hill, Chapel Hill, NC

**Background:** While complications of post-mastectomy breast reconstruction can affect the timing of adjuvant chemotherapy, chemotherapy can affect the wound healing process after reconstruction. Due to this interdependence, adequate communication between medical oncologists (MO) and plastic surgeons (PS) may be critical to optimize patient outcomes. In this study, we sought to evaluate the knowledge MO and PS have of each other’s fields, the roles they expect of each other, and how often they communicate in treating breast reconstruction patients.

**Methods:** A cross-sectional survey was conducted in a convenience sample of MO who treat at least one breast cancer (BC) patient per month and PS who perform at least one breast reconstruction per year. Surveys were distributed via email, US mail, and at two professional meetings. The survey included knowledge questions about reconstruction and chemotherapy, questions about provider and patient responsibilities for timely chemotherapy initiation, and questions about communication with other specialties. Fisher’s Exact and Wilcoxon Rank Sum tests were used to compare differences between groups.

**Results:** 53 medical oncologists and 23 plastic surgeons completed surveys (51% response rate). MO and PS had similar overall knowledge scores (MO 59%, PS 56%, p=0.5). 23% of MO knew the correct treatments for breast skin necrosis, and 60% knew the correct treatment for seromas. 32% of PS knew the approximate duration of Her2-directed therapy, and 14% of PS identified the usual adjuvant treatment for patients who have had neoadjuvant chemotherapy. Providers from both specialties who primarily practice in a rural setting scored significantly lower on the knowledge portion of the survey than those practicing in an academic medical center or urban/suburban area (51% rural versus 59%, p=0.03).

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**Conclusion:** There is increasing evidence of clinical equipoise between types of LD breast reconstruction and despite acknowledged cosmetic disadvantages the overall effects of PMRT on HRQL are minimal. The identification of important variables that may affect HRQL is crucial in all studies evaluating the effects of surgery on PROMS. Their integration into study results is essential for correct interpretation of clinically based assessments. This remains a challenging aspect in cohort studies, and emphasises the need for pragmatism in design of trials in the field.

than about uncomplicated patients (mean 3.9 times over first 6 month for complications versus mean 1.6 over first 6 months for uncomplicated). This translated to communicating about 2.5 times as often for complicated patients (p<0.001).

Conclusion: Medical oncologists and plastic surgeons have substantial deficits in knowledge about each other’s fields and differ in their opinion regarding the burden of responsibility in ensuring timely chemotherapy initiation. While MO and PS increase their communication when complications arise, these data suggest room for improvement in communication and understanding, which could improve the care of BC patients who undergo reconstruction and chemotherapy.

**P2-16-03**

Outcomes of Nipple-Sparing Mastectomy (NSM) and Immediate Reconstruction.

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**Background:** Nipple-sparing mastectomy (NSM) is the surgical removal of breast tissue that preserves the entire skin envelope including the nipple areola skin (NAS). We report our experience performing NSMs and immediate breast reconstruction for both high-risk breast cancer treatment and prophylaxis over a six-year period at The University of Utah and Huntsman Cancer Hospital.

**Methods:** A retrospective chart review was performed on patients undergoing NSM from April 2005 – April 2011. Data collection included: patient demographics, oncologic details, surgical information (including reconstruction timing and type), and complications (infection, hematoma, seroma, skin necrosis, NAS complication, skin flap loss, premature expander exchange/removal, and capsular contracture).

**Results:** 130 patients underwent 205 NSMs. Of these, 106 (81.5%) patients received mastectomy treatment for cancer while 24 (18.5%) patients were prophylactically treated. 102 NSMs (49.8%) were on breasts with biopsy-proven cancer, while 103 (50.2%) NSMs were on breasts for prophylaxis. All patients were female with a mean age of 44.7 years (range, 16 – 82 years). 119 (92.2%) patients were Caucasian, 3 (2.3%) were Asian, and 1 (0.8%) was Hispanic. The age of 44.7 years (range, 16 – 82 years). 119 (92.2%) patients were prophylactically treated. 102 NSMs (49.8%) were on breasts with cancer, while 103 (50.2%) NSMs were on breasts for cancer while 24 (18.5%) NSMs were for prophylaxis. Overall, complication rates are higher in breasts treated for cancer. Overall, complication rates are low in both cases of cancer and prophylaxis; this demonstrates that NSM and immediate reconstruction is a highly effective method of treatment for both groups.

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<tr>
<th>Complication</th>
<th>Cancer (n=102)</th>
<th>Prophylaxis (n=103)</th>
<th>Total</th>
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</tr>
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<tr>
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<tr>
<td>Capsular Contracture</td>
<td>13</td>
<td>14</td>
<td>27</td>
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**Conclusion:** When comparing NSMs in both patients and individual breasts with cancer to patients and breasts treated for prophylaxis, there is no significant difference in complication rates by case or breast, except for the capsular contracture rate, which was significantly higher in breasts treated for cancer. Overall, complication rates are low in both cases of cancer and prophylaxis; this demonstrates that NSM and immediate reconstruction is a highly effective method of treatment for both groups.

**P2-16-04**


Ochoa O, Chrysochou M, Nastala C, Ledex P, Pisan S. Plastic Reconstrucive and Microsurgical Associates of South Texas, San Antonio, TX

Introduction: Promoted by reports of decreased donor-site morbidity compared to traditional transverse rectus abdominis (TRAM) flap breast reconstruction, deep inferior epigastric perforator (DIEP) flaps have gained significant popularity. Increasing body mass index (BMI) is associated with poor flap-specific and donor-site outcomes in breast reconstruction using traditional techniques. The current study aims at defining flap-specific and donor-site complications with increasing BMI in patients undergoing DIEP flap breast reconstruction.

**Methods:** A retrospective analysis of 639 DIEP flaps in 418 consecutive patients between Jan 2006 to March 2008 was performed. Patients were stratified into five groups based on BMI: normal weight (≤ 24.9), overweight (25-29.9), obese (30-34.9), severely obese (35-39.9), and morbidly obese (≥ 40). Medical co-morbidities, adjuvant chemo-radiation therapy, timing of reconstruction, active tobacco use and surgical history were collected. Primary outcomes were compared between BMI groups.

<table>
<thead>
<tr>
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<td>NAS Complication</td>
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<td>1</td>
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<td>1.00</td>
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<tr>
<td>Skin Flap Loss</td>
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<tr>
<td>Capsular Contracture</td>
<td>13</td>
<td>6</td>
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<td>0.39</td>
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</table>

**Conclusion:** When comparing NSMs in both patients and individual breasts with cancer to patients and breasts treated for prophylaxis, there is no significant difference in complication rates by case or breast, except for the capsular contracture rate, which was significantly higher in breasts treated for cancer. Overall, complication rates are low in both cases of cancer and prophylaxis; this demonstrates that NSM and immediate reconstruction is a highly effective method of treatment for both groups.
thrombotic and overall complications including flap failure were not significantly different between groups. Donor-site complications stratified by BMI demonstrated significantly increased delayed wound healing and overall complications among morbidly obese patients compared to other groups. Incidence of donor-site infection, acute or subacute hematomas, seroma formation, abdominal wall bulging, and hernia formation were not significantly different between groups despite increasing BMI.

Conclusion: Increasing BMI predisposes patients to delayed wound healing complications in both flap and donor-site locations. Nevertheless, overall flap complications remain similar across all BMI groups. Despite increased overall donor-site complications in patients with increasing BMI, abdominal wall stability was maintained during a follow-up period approximating 3 years. Given a similar flap complication profile and maintenance of abdominal stability, DIEP flaps are recommended in patients with increased BMI.

**P2-16-05**

**Delay of Adjuvant Chemotherapy after Elective Mastectomy and Immediate Reconstruction in Breast Conservation Candidates: A Matched-Pair Analysis.**

Barry PN, Riley EC, Pan J, Crew JB, Lee K, Jain D, Kruse B, Quillo AR, Rai S, Dragan AE. James Graham Brown Cancer Center; University of Louisville School of Medicine, Louisville, KY

Background: To analyze factors that influence the timing of adjuvant chemotherapy in patients who are candidates for breast conservation therapy (BCT) but elect mastectomy with immediate reconstruction (M-IR).

Methods: Using data from the University of Louisville Cancer Registry, we identified 35 consecutively-treated patients with stage I or II breast cancer between 2004 and 2009 who underwent M-IR and adjuvant chemotherapy. These were matched for age and AJCC stage to 35 controls who underwent BCT and adjuvant chemotherapy. We examined the timing of initiation of chemotherapy from the date of surgery and assessed the probability of therapeutic delay using univariate logistic regression and McNemar’s test for matched pairs.

Results: For the 70 patients included in this study, the median age was 46y (range: 30-65y), and the distribution for stage I, IIa and IIb was 22.9%, 65.7% and 11.4%, respectively. The two groups were well balanced in terms of race, rural/urban status, smoking, diabetes, insurance coverage, and histology. The median time to initiation of adjuvant chemotherapy was 38 days (range: 25-103 days) for BCT and 55 days (range: 30-165 days) for M-IR. Patients undergoing M-IR were more likely to experience any delay (> 45 days; 74.3% vs. 25.7%, p < 0.001) and/or significant delay (>90 days; 20.0% vs. 2.9%, p < 0.001) than those choosing BCT. On univariate logistic regression analysis, the extent of surgery had a major impact on the likelihood of any delay in chemotherapy (OR= 8.35, 95% C.I. = 2.86-24.3, p <0.001). None of the other aforementioned factors predicted for delay.

Conclusion: The use of elective mastectomy with immediate reconstruction in breast conservation candidates independently predicts for delay in initiation of adjuvant chemotherapy. Further study is needed to qualify the underlying causes of and ultimate clinical significance of these delays.

**P2-16-06**

**A Systematic Review of Standardised Clinical Outcomes and Patient Reported Outcome Measures (PROMS) in Breast Reconstruction.**

Winters ZE, Chaudhry A, Benson JR. University Hospitals Bristol NHS Foundation Trust, Bristol, South West, United Kingdom; Cambridge University Hospitals NHS Foundation Trust, Cambridge, East Anglia, United Kingdom

**Introduction**

Failure to prospectively define and report surgical complications across a range of study designs is a confounding factor that fundamentally compromises the interpretation of data on health-related quality of life (HRQL) and PROMS. The UK National Mastectomy and Breast Reconstruction audit on 5000 breast reconstruction patients found higher than expected levels of complications (including rates of reoperation and levels of infection). We aimed to analyze the reporting of complications in a systematic review of all studies evaluating PROMS in breast reconstruction since 1978.

**Methods**

Efficace criteria for HRQL methodology were used to assess the inclusion eligibility for each study. Two out of 34 eligible studies were excluded due to overlapping patient datasets and small numbers (n<=5) of breast reconstruction cases. Two reviewers examined the reporting of complications. The study designs comprised: 2 randomised controlled trials (RCT); 10 prospective longitudinal studies (PLS) and 20 retrospective studies (RS). RCTs and PLSs were evaluated for predefined complications, their grading according to Clavien-Dindo and their integration into the evaluation of PROMS results. RS were evaluated for the reporting of complications and their statistical methodology.

**Results**

A total of 3213 patients were included in the 32 studies. The type of reconstruction was only recorded in 71% of patients. The majority of patients in these studies underwent abdominal flap reconstruction, compared to implant only techniques (32%), and less than 10% comprising Latissimus dorsi flaps. Only 19% of studies recorded complications with the majority (81%) failing to report any complications. Only 3 studies provided details of the numbers and level of complications amongst a total of 44 patients (1.4% of the total number of patients). Reported complications were graded and classified either as major (requiring surgical intervention) or minor comprising 55% and 45%, respectively. None of the RCT or PLS studies defined either the type or level of complications a priori, nor stratified complications based on risk factors such as age, body mass index, smoking and diabetes.

**Conclusion**

There is a significant under estimation of the contribution of complications to HRQL and PROMS reporting. This fact diminishes the current evidence on the effects of breast reconstruction on PROMS. There should be collective efforts to improve the standards of documentation for clinical outcomes in breast reconstruction. This together with standardized reporting of PROMS will consolidate clinical evidence upon which decision-making and provision of patient information can be confidently based.

P2-16-07
A Randomised Controlled Trial To Evaluate the Role of Tisseel, a Fibrin Sealant on Seroma Formation in Latissimus dorsi Breast Reconstruction.
Winters ZE, Llewellyn-Bennett R, English R, Turner J, Rayter Z, Greenwood R. University Hospitals Bristol NHS Foundation Trust, Bristol, South West, United Kingdom

Introduction
Donor site seromas are common complications following Latissimus dorsi breast reconstructions (LDBR), as shown in the UK National Mastectomy and Breast Reconstruction audit. Level I clinical evidence following the performance of an RCT supports ‘fixation’ of the back skin flaps by quilting sutures. Fibrin sealants (Tisseel) have been postulated to reduce seromas after simple mastectomy and axillary dissections, but their role in reducing donor site seromas after breast reconstruction has yet to be performed in a ‘robust’ clinical trial. The aim of this RCT was to compare Tisseel against Control (no Tisseel) on the incidence of seromas after LDBR.

Methods
In an ethics approved single centre, single-blinded study comprising 2 surgeons from 2005-2010; 106 women were randomised to either Control (52) or Tisseel (54) interventions after immediate or delayed breast reconstructions. Sixteen patients were excluded as follows due to incomplete data at 3 months or re-operations of the donor site for complications. The types of breast reconstructions comprised implant-assisted LD (LDI) in 45 women, 23 extended LD flaps (ELD) and 23 ELD with implant (ELDI). Immediate breast reconstructions (n=87) comprised the majority compared to only 4 delayed procedures. Intraoperative drains were placed to the breast, axilla and donor site (x two) as per standard practice. A 0.5% fibrinogen concentration was used in a hydraulic hand-held Tisseel spray application to the donor site chest wall over 60 seconds. Two stay sutures were pre-placed 2 cm adjacent to the donor wound skin edges above and below prior to the Tisseel application. In the control group, 2 drains only were placed.

The primary outcome measure was the total seroma volumes from all the sites over 3 months. This was used for the power calculation of the sample size and showed a requirement for a minimum of 95 women. Secondary outcomes included the volumes of the donor site seromas, and the frequency of post-drain removal donor site aspirations of all symptomatic seromas by patient self-report.

Results
The effect of Tisseel glue was to reduce the mean total drain (breast, axilla and back) volume from 2170ml to 1919ml (P=0.05, Mann-Whitney) within 7-10 days. The donor site seromas volumes were similar between the 2 groups over 3 months. The mean donor site total drain volumes (LD donor site drain volume and symptomatic donor site aspirations) were 5412ml in the Control group (840-6252), compared to the Tisseel group producing 4646ml (5384-738). There were no statistical differences between the frequencies or volumes of patient reported seromas aspirated post-drain removal between the two groups. This comprised a mean of 4 aspirations (range 0-13) in the Control group compared to a mean of 9 aspirations (range 0-11) after the use of Tisseel (P=0.548).

Conclusion
Tisseel glue may reduce the ‘early’ effect of seroma development, but has not shown any significant role in minimising the potential ‘shearing’ of the donor site skin flaps causing later seroma formation. Current evidence recommends quilting sutures as the gold-standard in reducing this complication.
2. Jain PK et. al. BJS 2004; 91:54-60.
P2-16-09
Gath AA, Blechman K, Levowitz C, Small K, Axelrod D, Karp N, Choi M. NYU-Langone Medical Center, New York, NY; NYU-Langone Medical Center

Purpose: Nipple and/or areola-sparing mastectomy as a therapeutic or prophylactic procedure for breast cancer is rapidly gaining popularity as the literature continues to support it safety. The lateral inframammary fold (IMF) approach provides adequate exposure and eliminates visible scars on the anterior surface of the breast, making this incision cosmetically superior to radial or periareolar approaches.

Methods: We reviewed 58 consecutive nipple and/or areola-sparing mastectomies performed through a lateral IMF incision with immediate implant-based reconstruction, with or without tissue expansion, between June 2008 and February 2011. Prior to incision, breasts were lightly tumesced with dilute anesthetic solution with epinephrine. Sharp dissection, rather than electrocautery, was used as much as possible to minimize thermal injury to the mastectomy flap. When indicated, acellular dermal matrix was placed as an inferolateral sling. Subsequent fat grafting to correct contour deformities was performed in select patients. Three-dimensional (3D) photographs assessed changes in volume, antero-posterior projection, and ptosis. Retroareolar/nipple tissue underwent routine intraoperative frozen section analysis in cancer cases.

Results: Mean patient age was 44 years, and mean follow-up time was 14 months. Depending upon the judgment of the oncologic surgeon, 44 (76%) mastectomies were nipple/areola-sparing, and 14 (24%) were areola-sparing. Thirteen mastectomies (22%) were therapeutic, the remaining 45 mastectomies (78%) were prophylactic. Five of the nine sentinel lymph node biopsies (56%) were performed through the lateral IMF incision without the need for a counter-incision. Acellular dermal matrix was used in 44 (76%) breasts. Average permanent implant volume was 313 cc (range 170 to 750 cc), and average fat grafting volume was 90 cc (range 36 to 177 cc). Mastectomy flap necrosis, requiring operative debridement, occurred in three breasts (5%). One of these breasts required a salvage latissimus dorsi myocutaneous flap to complete the reconstruction. Of the 44 nipple/areola sparing mastectomies, three (7%) required operative debridement and reconstruction for partial nipple necrosis. No statistically significant differences existed between therapeutic and prophylactic mastectomies for developing partial skin or nipple necrosis (p = 0.65). Morphologic outcomes using 3D scan measurements showed reconstructed breasts were larger, more projected, and less ptotic (196 vs. 248 cc, 80 vs. 90 mm, 146 vs. 134 mm, p < 0.01 for each parameter).

Conclusion: Excellent results can be achieved with immediate implant-based reconstruction of nipple and/or areola-sparing mastectomy through a lateral IMF incision. NAC survival is reliable, and complication rates are low.

P2-16-10
Practice Variations in Post-Mastectomy Breast Reconstruction: What Are the Roles of Clinical Factors, Access Barriers, and Delayed Reconstruction?
Cox D, Milickey J, Dominik R, Lee C. University of North Carolina at Chapel Hill, Chapel Hill, NC

Background: Breast reconstruction rates vary widely by race, income, and location. Proposed explanations have included clinical contraindications to reconstruction, access barriers, and patient or surgeon preference for delayed reconstruction. We sought to determine whether clinical contraindications (planned radiation, comorbidities) or access barriers (distance to care, surgeon) were associated with reconstruction and to estimate the frequency of delayed reconstruction over time.

Methods: A retrospective cohort study was conducted among women treated with mastectomy for Stage 0-IIA breast cancer between 2002 and 2007 at one institution. Demographics, stage, comorbidities, adjuvant therapy plans, and treatments were obtained from the medical record. Multivariable modified Poisson regression analyses were performed, with immediate and any reconstruction (immediate or delayed) as outcomes. Interaction terms were added to examine surgeon effects. Kaplan-Meier survival analysis was performed.

Results: Among 539 women, 18.9% had a plan for chemotherapy; 13.7% had a plan for radiation. Comorbidities were: obesity 17.1%, diabetes 11.9%, smoking 16.3%, heart disease 5.6%, immunodeficiency 2.2%. Most patients (59.8%) lived within 50 miles of the hospital. 33.8% had immediate reconstruction; 5.6% had delayed. Patients who had a plan for adjuvant therapy or comorbidity were less likely to have reconstruction. Patients who lived farther from the hospital were slightly more likely to have reconstruction, but this was not statistically significant (p<0.08). None of the associations varied significantly by surgeon. Few patients had reconstruction more than 2 years after mastectomy.

Multivariable Poisson regression analysis of factors associated with immediate or any reconstruction

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</thead>
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<td>Age</td>
<td>RR 95% CI</td>
<td>RR 95% CI</td>
</tr>
<tr>
<td>Plan for adjuvant therapy</td>
<td>0.43 0.26, 0.64</td>
<td>0.59 0.45, 0.79</td>
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<tr>
<td>Comorbidities</td>
<td>0.67 0.56, 0.80</td>
<td>0.67 0.56, 0.79</td>
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<tr>
<td>Distance&gt;50 miles</td>
<td>1.17 0.92, 1.45</td>
<td>1.13 0.94, 1.33</td>
</tr>
</tbody>
</table>

Conclusion: Clinical contraindications may explain some breast reconstruction practice variations. Few patients who defer immediate reconstruction, however, ever have delayed reconstruction.

P2-16-11
Role of Proper Patient Counselling in Combination with Effect of Socioeconomic Deprivation on the Rate of Immediate Breast Reconstruction after Mastectomy.
Chakrabarti M, Stallard S, Fitzgerald C, Obondo C, Weiler-Mithoff E, Doughty J, Romans, Jr L. Victoria Infirmary Glasgow; Glasgow Royal Infirmary; Western Infirmary Glasgow

Introduction: current guidelines in the United Kingdom suggest that the possibility of breast reconstruction should be discussed with all patients prior to mastectomy. However, the majority of patients are still treated with mastectomy alone and no reconstruction is carried out. It has also been suggested that women from more deprived areas are less likely to undergo immediate breast reconstruction (IBR). We investigated potential pitfalls in patient counselling and consequent decision making contributing to present IBR rate in combination with the effect of socioeconomic deprivation.

Methods: data from 89 consecutive mastectomy patients was prospectively collected in a single centre in Glasgow between August 2010 and March 2011. Each patient was scored for deprivation based on The Scottish Index of Multiple Deprivation. The patients were then divided into two groups: high and low deprivation levels. Consultations about IR and patients’ acceptance of counselling were analysed. For statistical calculations Fischer’s exact test was applied. Results: IBR was offered to 41 (46%) patients, but it was not to 42 (47%) (6 were excluded due to incomplete data). 25 patients accepted IBR, and of those 24 (27%) underwent IBR. 16 of 41 patients refused to undergo IBR due to lack of interest (10), not feeling ready for it (2), preference of delayed procedure (2) and fear of delaying
adjuvant therapy (2). Of 42 patients whom IBR was not offered, only 10 were documented in the notes, while there was no reference for discussing reconstruction in 32 (76%) cases. Reasons for not even discussing reconstruction were the following: age (15), co-morbidities (18), locally advanced cancer (2), co-morbidities with age (5), and locally advanced cancer with age (2). As regards to socioeconomic deprivation; 44 (49%) patients were from deprived areas and 39 (44%) from affluent areas. 41 patients were offered IBR and of these 23 (26%) were from affluent areas compared to 18 (20%). Of the 42 patients who were not offered IBR, 26 (29%) were from deprived while 16 (18%) from affluent areas (p<0.05). Of the 44 deprived patients, 18 were offered IBR but 26 were not. 15 of 25 patients, who accepted IBR, were from affluent areas. The 16 patients who refused IBR had equal distribution of deprivation.

Conclusions: while none of the reasons for not offering IBR represent absolute contraindication to IBR, decisions about refusal are based mostly on patients’ subjective intuitions. Further, a greater proportion of the patients who were not offered IBR were from more deprived areas, and it seems that patients from affluent areas are more likely to be offered and IBR compared to ones from deprived areas. However, confounding factors such as co-morbidities may contribute to the above. We believe, therefore, that detailed counselling about reconstruction of each patient requiring mastectomy is necessary, which is likely to further increase IBR rate.

<table>
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<tr>
<td>IBR offered: 41 patients (46%)</td>
<td>18 (20%)</td>
<td>23 (26%)</td>
</tr>
<tr>
<td>IBR not offered: 42 patients (47%)</td>
<td>26 (29%)</td>
<td>16 (18%)</td>
</tr>
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<td>(Total 83, excluding 6 patients)</td>
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</tbody>
</table>

**P2-16-12**

Skin Reducing Nipple Sparing Mastectomy – A Treatment Option for Patients with Significant Ptois or Macromastia.

Ott Young A, Alizadeh K, Hodyl C, Pronovost M, Davenport T. Yale New Haven Health System, Fairfield, CT; Long Island Plastic Surgical Group, Garden City, NY; South Nassau Communities Hospital, Oceanside, NY

Background: With new data showing the oncologic safety of nipple sparing mastectomy with immediate reconstruction - nipple sparing mastectomies are now offered to an increasing number of mastectomy patients. Unfortunately cosmetically acceptable reconstruction of nipple sparing mastectomies is technically limited to women with perfect breast anatomy - where the skin envelope shows no or little redundancy and where the nipple areolar complex is located in the perfect location on the breast mount. Especially in countries with a high incidence of obesity and associated macromastia only a small percentage of women fall into this category. A high percentage of patients who would otherwise be good candidates for nipple sparing procedures are excluded based on their unfavorable breast anatomy. We describe a surgical technique that reduces the redundant mastectomy skin envelope in a breast reduction pattern and places the native nipple areolar complex as a free skin graft into proper anatomical position. This technique in conjunction with implant or autologous reconstruction combines the benefits of shorter better vascularized skin flaps with the cosmetic and emotional benefits of preserving the nipple areolar complex even in the anatomically difficult cases.

Methods: To investigate the safety and efficiency of this method we reviewed medical records of 40 patients who underwent 60 nipple preserving skin reducing mastectomies over the last 4 years. All procedures were performed by the senior author and follow up ranged from 4 weeks to 50 months.

The charts were analyzed for the occurrence of complications such as infection, seroma, hematoma, mastectomy flap necrosis, nipple-areolar loss and loco regional recurrence. All patients were asked to rate their reconstruction on a scale of poor-good-excellent.

Results: In 60 reconstructed breasts we encountered one case of seroma, four cases of limited mastectomy flap necrosis treated with local wound care and one case of partial areolar necrosis treated with local excision. No loco regional recurrences, hematomas or infections were encountered. Out of the group of 40 patients, 31 patients rated their result as excellent, 8 as good, and one as poor.

Conclusion: Our results compare favorably with data published in the literature. Patient satisfaction rates are high and complication rates are low in a patient population that historically has been a challenge for the breast surgeon, as well as, the plastic surgeon. The use of a skin reducing mastectomy in conjunction with a free nipple graft enables the breast surgeon to offer nipple sparing procedures for all patients who are good oncologic candidates regardless of their breast anatomy.

**P2-16-13**

Life after Mastectomy without Breast Reconstruction – Are the Needs of Caribbean Women Different?

Morton-Gittens J, Ali R, Ajfan AM, Bovell P, Rampaul R. National Radiotherapy Centre, St James, Trinidad and Tobago

Aim: The standard of care in the United States and United Kingdom for women undergoing mastectomy is to be offered breast reconstruction surgery (BRS), either immediate or delayed. In Trinidad, for a variety of reasons, patients are not routinely offered this option. We undertook a study to determine the impact of breast surgery without reconstruction on their perception of sexuality, body image, relationships and quality of life. The aim is to establish a benchmark for the planning and incorporation of reconstructive options into the care pathway of patients requiring oncoplastic surgery in the Caribbean.

Method: A questionnaire was administered to post surgery breast cancer patients in a public oncology clinic in Trinidad. These patients had no BRS and had surgery at various institutions in Trinidad, where the primary surgical service was General Surgery. Data was collected prospectively and analysed using SPSS software.

Results: Of 130 questionnaires administered to date, 80(61.5%) of those collected were adequate for analysis. 46.1%(n=35) of patients were less than fifty years of age; 44%(n=24) were Afro-Caribbean and 55.6%(n=30) East Indian-Caribbean; 61.1%(n=44) had mastectomies and 38.9%(n=28) had breast conserving surgery (BCS). Of all patients, 90%(n=70) felt attractive, feminine and satisfied with their bodies before surgery, however postoperatively, all who had BCS remained satisfied with their bodies but a significant proportion of patients in the mastectomy group were dissatisfied (p=0.006) and found it harder to look at themselves naked (p=0.025) and be seen naked by their partners (42.9%, n=15). East Indian-Caribbean women felt less attractive after surgery compared to Afro-Caribbean patients (p=0.024). Among all patients, 19.2% felt shy to go out in public after surgery, 28.8% left their house less and 35% (n=25) had fewer social interactions. When analysed by ethnicity, East Indian-Caribbean women were more likely to avoid public events (p=0.003) and had fewer social interactions (p=0.028) than Afro-Caribbean patients. This effect persisted in mastectomy patients (p=0.007), especially in the East Indian-Caribbean group (p=0.0017). 69% of
patients were willing to undergo BRS, but it was offered to only 32.5% (n=13) of mastectomy patients. The interest in BRS was similar in all races, age groups and surgery types.

Conclusion: This study explores the experiences and needs of Caribbean women with breast cancer in Trinidad. We found from this data that their needs mirror those of women in other countries where BRS is incorporated into the core care pathway. Our data demonstrates that even with ethnic differences, there is deterioration in self esteem and femininity with breast cancer surgery, with lasting effects. Subtle differences appear to be present in body image perception amongst East Indian-Caribbean women versus Afro-Caribbean and may be a useful template when assessing patient needs with a view to how we individualize care in the Caribbean setting. This data also emphasizes the potential benefit in incorporating an Oncology-Plastic Surgery Team approach in our setting and supports the view that providing BRS to patients as early as possible can help to maintain a positive outlook in personal and social interactions.

P2-16-14
Skin Sparing Mastectomy and Immediate Latissimus Dorsi Flap Reconstruction: Patient Reported Outcome and Factors Affecting the Highest Patient Satisfaction.
Kim Z, Kang S-G, Lee J, Kim SY, Lim CW, Lee MH. Soonchunhyang University Hospital, Seoul, Korea

Background: Skin sparing mastectomy (SSM) and immediate breast reconstruction (IBR) with latissimus dorsi (LD) flap is a tailored surgical procedure for breast cancer patients. In this study the oncologic safety, morbidities, and aesthetic results of SSM and LD IBR, as regards to patient satisfaction, were assessed.

Material and Methods: Between March 2000 and February 2011, single surgeon performed SSM and IBR for 145 patients. Eighty-five patients completed the patient satisfaction survey, and 65 patients with SSM and LD IBR were included. The patients were divided into 2 groups according to their degree of satisfaction, and a stratified analysis was performed.

Results: The mean age of the patients was 48.4 years (range, 21-74), and the pathologic results were infiltrating ductal carcinoma (n=48, 73.8%), ductal carcinoma in situ (n=15, 23.1%), and others (n=2, 3.1%). After a mean follow-up of 34 months (range, 1.6-89.9) no local recurrence occurred, and there were no skin necrosis or LD flap loss. Donor site morbidities were seroma (n=8, 12.3%), scarring (n=8, 12.3%), and back pain (n=6, 9.2%).

Table: Post-operative complications and morbidities

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group 1 (n=39)</th>
<th>Group 2 (n=26)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematoma</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Infection</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Skin flap complication</td>
<td>0</td>
<td>0</td>
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</tr>
<tr>
<td>Skin necrosis</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Donor site complication</td>
<td>0.15 (4.4%)</td>
<td>2 (7.7%)</td>
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</tr>
<tr>
<td>Seroma</td>
<td>4 (10.3%)</td>
<td>2 (7.7%)</td>
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</tr>
</tbody>
</table>

Conclusion: The present study demonstrated that our SSM and LD IBR was oncologically safe, and was associated with low morbidity rates. LD flap per se, without a prosthesis implant, could sufficiently produce a high level of patient satisfaction. Accurate surgical techniques and careful consideration for breast symmetry and nipple cosmesis would be the key to meet the patient’s expectations and enhanced quality of life.

P2-16-15
Oncological Safety and Survival Rate According to Reconstructive Surgery in Advanced Breast Cancer after Neoadjuvant Chemotherapy.
Kim WW, Lee JJ, Nam KH, Jung JH, Chae YS, Yang JD, Lee YH, Park HY. Kyungpook National University; Hyesung Hospital

Purpose
Oncoplastic surgery has received attention according as the incidence of breast cancer is rising and quality of life including cosmetic result after surgery is regarded as important. The aim of this study is to compare combined reconstructive surgery with standard surgery regarding to oncological safety and survival rate in advanced breast cancer after neoadjuvant chemotherapy (CTx).

Method
Thirty-seven patients underwent neoadjuvant CTx and surgery with advanced breast cancer were analyzed from September 2007 to March 2010. Group A (n=12) received combined reconstructive surgery, group B (n=25) had standard surgery.

Results
There were no differences in age, size, metastatic LN, stage, ER/PR/Her-2 status, recurrence, metastasis and death between group A and B. Patients with good response in neoadjuvant chemotherapy (26 cases (72.9%)) had CTx-operation-radiation therapy (RTx), cases with poor response (10 cases (27.1%)) underwent CTx-RTx-operation. There was significant difference in order of treatment, eight patients among group A(66.6%) had CTx-RTx-operation, 22 cases in group B(88.0%) received CTx-operation-RTx (p=0.006). Mean follow up period was 22 months, 2 patients (5.2%) experienced local recurrences, 11 cases (34.3%) diagnosed with distant metastasis, and 4 patients (10.5%) expired with breast cancer.

Table: Aesthetic results

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group 1 (n=39)</th>
<th>Group 2 (n=26)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin incision</td>
<td></td>
<td></td>
<td>0.241</td>
</tr>
<tr>
<td>Circum-areolar</td>
<td>32 (82.1%)</td>
<td>24 (92.3%)</td>
<td></td>
</tr>
<tr>
<td>Circum-areolar + extension</td>
<td>7 (17.9%)</td>
<td>2 (7.7%)</td>
<td></td>
</tr>
<tr>
<td>Contralateral procedure</td>
<td></td>
<td></td>
<td>0.334</td>
</tr>
<tr>
<td>No</td>
<td>38 (97.4%)</td>
<td>24 (92.3%)</td>
<td></td>
</tr>
<tr>
<td>Yes (reduction)</td>
<td>1 (2.6%)</td>
<td>2 (7.7%)</td>
<td></td>
</tr>
<tr>
<td>Nipple reconstruction</td>
<td></td>
<td></td>
<td>0.128</td>
</tr>
<tr>
<td>No</td>
<td>18 (46.2%)</td>
<td>17 (65.4%)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>21 (53.8%)</td>
<td>9 (34.6%)</td>
<td></td>
</tr>
<tr>
<td>Donor site scar length, cm</td>
<td>17.5±2.8 (7.0-24.0)</td>
<td>17.2±1.8 (13.6-22.0)</td>
<td>0.788</td>
</tr>
<tr>
<td>Breast size symmetry</td>
<td>6.9±2.0 (3.0-10.0)</td>
<td>8.7±1.4 (5.0-10.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Nipple cosmesis</td>
<td>7.7±1.6 (3.0-10.0)</td>
<td>9.0±0.9 (6.0-10.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Surgeon assessment</td>
<td>6.9±2.1 (3.0-10.0)</td>
<td>8.8±1:3 (5.0-10.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Breast clinic nurse assessment</td>
<td>7.2±2.0 (3.0-10.0)</td>
<td>6.1±1.0 (6.0-10.0)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Group 1: poor, fair, and good patient satisfaction. Group 2: excellent patient satisfaction

Conclusion: This study explored the experiences and needs of Caribbean women with breast cancer in Trinidad. We found from this data that their needs mirror those of women in other countries where BRS is incorporated into the core care pathway. Our data demonstrates that even with ethnic differences, there is deterioration in self esteem and femininity with breast cancer surgery, with lasting effects. Subtle differences appear to be present in body image perception amongst East Indian-Caribbean women versus Afro-Caribbean and may be a useful template when assessing patient needs with a view to how we individualize care in the Caribbean setting. This data also emphasizes the potential benefit in incorporating an Oncology-Plastic Surgery Team approach in our setting and supports the view that providing BRS to patients as early as possible can help to maintain a positive outlook in personal and social interactions.
Conclusion
Advanced breast cancer with poorly responded in neoadjuvant CTx could be actively treated with sequential CTx-RTx-reconstructive surgery. Combined reconstructive surgery was oncologically safe operation in advanced breast cancer.

P2-16-16
Nipple Sparing Mastectomy and Immediate Breast Reconstruction: Critical Appraisal of Five Year, Single Centre Outcomes.
Westbroek D, Karat I, Min-Hui H, Gukas I, Daoud R, Laidlaw IJ. Frimley Park Hospital, Portsmouth Road, Frimley, Surrey, United Kingdom

Background:
Approximately 5,000 patients undergo breast reconstruction annually in the United Kingdom (2:1 ratio of immediate versus delayed respectively). Whilst skin sparing mastectomy is an accepted modality for local control in appropriately selected patients, nipple preservation remains controversial. In over 1,800 peer reviewed nipple sparing mastectomy (NSM) cases, the local event rate attributable to NAC preservation following NSM (therapeutic and prophylactic) is 0.16% with no evidence of deleterious impact on disease free survival. This study aims to evaluate in a selected cohort of patients, the oncological safety and aesthetic outcome of nipple- sparing mastectomy and immediate breast reconstruction (IBR). We highlight the surgical technique used and propose in/exclusion criteria.

Material and Methods:
Patients were identified retrospectively from our Institution’s electronic patient records and WinPath™ database by searching clinical codes corresponding to NSM & IBR, performed by the senior authors (IJL and RD) between January 2004 and December 2008. Pre-operative selection criteria included: 100% pre-operative lesion characterisation; 5mm margin acceptance; submission of separate nipple core specimens for paraffin block histological assessment and re-excision of all involved/ close margins. Follow-up data was verified from patients’ case notes, histo- pathological review and standardised photographic views. Endpoints include: local recurrence rate, disease free survival, peri-operative NAC subcutaneous tissue loss and validated aesthetic outcome measures.

Results:
84 patients underwent NSM & IBR for invasive disease. The mean age was 52.1 years and a median follow-up of 38 months. IBR utilised autologous tissue (latissimus dorsi or transverse rectus abdominus musculocutaneous flaps) with/ without implant prosthesis. There was one case of local recurrence (1.2%) in a patient who declined re-excision of close/involved margins and no recorded breast cancer related deaths. Peri-operative complication rates were within the national mastectomy breast reconstruction audit outcome guidelines.

Conclusion:
In this cohort of patients, nipple sparing mastectomy and immediate breast reconstruction achieves comparable oncological outcomes with excellent aesthetic relative to traditional ablative procedures for local control. Nipple preservation although not evaluated independently does enhance cosmetic outcomes.

*(Preliminary analysis)

P2-17-01
Coleman R, De Boer R, Eidiathmann H, Neven P, von Minckwitz G, Martin N, Modi A, Bandred N. University of Sheffield, Sheffield, United Kingdom; Royal Melbourne Hospital, Victoria, Australia; University Frauenklinik, Kiel, Germany; UZ Gasthuisberg, Leuven, Belgium; German Breast Group, Frankfurt, Germany; Novartis Oncology; East Hanover, NJ; University Hospital of South Manchester NHS Foundation Trust, Manchester, United Kingdom

Introduction: Bisphosphonates (BPs) combined with adjuvant endocrine therapy have been shown to prevent aromatase inhibitor-associated bone loss (AIBL) and improve outcomes in recent clinical trials in patients with hormone receptor-positive (HR+) early breast cancer (EBC). Accelerated bone turnover (a common phenomenon during AIBL) has been associated with increased risk of bone metastasis in EBC (Lipton A, et al. St. Gallen 2009. abs #224), but little is known about the effect of BPs on the disease course in women with EBC and progressing AIBL. We have previously demonstrated that adding ZOL to adjuvant therapy significantly improved bone mineral density (BMD) and prolonged disease-free survival (DFS) vs delayed ZOL (de Boer R, et al. SABCS 2010. P5-11-01). We report here the prognostic factors for DFS and the effect of ZOL initiation in the delayed ZOL (DZOL) arm of the ZO-FAST trial at 5 years’ median follow-up.

Methods: The ZO-FAST trial randomized postmenopausal women with HR+ EBC initiating letrozole (LET; 2.5 mg qd × 5 years) with a BMD T-score ≥–2 (N=1,065) to immediate (IMZOL; 4 mg q 6 months) or DZOL (initiated for post-baseline T-score <–2, or nontraumatic/asymptomatic fractures). The primary endpoint was change in BMD at 12 months; patients were followed for disease recurrence and overall survival for 5 years (secondary endpoints). Exploratory Cox regression analyses were performed to identify prognostic factors for DFS in the DZOL arm.

Results: At 60-months follow-up, IMZOL significantly reduced the risk of a DFS event by 34% vs DZOL (42 vs 62 events; >80% distant recurrences and 20% local; hazard ratio [HR]=0.66; 95% confidence interval [CI], 0.44-0.97; P=.034) in the intent-to-treat population (N=1,065; n=532 IMZOL; n=533 DZOL). In exploratory analyses of the DZOL arm (n=535; safety population), patients who initiated DZOL treatment (n=144) had significantly improved DFS (10 events; HR=0.462; 95% CI, 0.23-0.94; P=0.033) compared with DZOL arm patients who never initiated ZOL (53 events; n=391). Other significant prognostic factors for DFS events in the DZOL arm were age ≥65 years at study entry (HR=1.949; 95% CI, 1.09-3.47; P=.024 vs age <65 years) and cancer T-stage ≥2 (HR=2.155; 95% CI, 1.03-4.51; P=.042 vs T-stage <2).

Conclusions: Exploratory analyses of the ZO-FAST database revealed significant DFS benefits from initiation of ZOL treatment for post-baseline fractures or T-scores <–2, suggesting that ZOL (even if initiated late) can positively influence the disease course in patients with AIBL. Together with other studies showing DFS benefits from ZOL in patients with complete ovarian suppression/postmenopausal status (Coleman RE, et al. SABCS 2010. abs #S4-5; Gnant M, et al. NEJM 2009;360:679-691), these data suggest that treating (and ideally, preventing) AIBL may also improve DFS in patients with HR+ EBC.
P2-17-02
Increased Progression Free and Overall Survival in Breast Cancer Patients with Menopausal Symptoms or Arthralgia/Myalgia during Adjuvant Treatment with Exemestane or Tamoxifen – Results of the German TEAM Trial.

Hadjii P, Kieback DG, Hasenburg A, Tams J, Ziller M. Phillipps University, Marburg, Germany; Elbländkliniken, Meissen, Germany; University Hospital Freiburg, Freiburg, Germany; ICRC-Weyer GmbH, Berlin, Germany

Background:
Previous studies have suggested a correlation between the occurrence of vasomotor or joint symptoms during tamoxifen or aromatase inhibitor treatment and improved clinical response. We assessed if there was any correlation between treatment-emergent adverse events during exemestane or tamoxifen treatment and clinical response.

Methods:
A retrospective analysis of the German cohort of the TEAM trial was performed to assess progression-free survival and overall survival in patients with and without menopausal symptoms or arthralgia/myalgia during adjuvant treatment with exemestane or tamoxifen.

Results:
A total of 1502 patients were included in this analysis; 739 patients received tamoxifen and 763 received exemestane. Patients reporting menopausal symptoms and patients reporting arthralgia/myalgia during tamoxifen or exemestane treatment had significantly longer overall survival and progression-free survival. The effect on overall survival was irrespective of treatment. Progression-free survival was significantly improved in exemestane-treated patients reporting menopausal symptoms or those reporting arthralgia/myalgia versus those not reporting these adverse events. There was no significant difference in progression-free survival between tamoxifen-treated patients reporting these symptoms versus those who did not. A combined analysis of patients reporting either menopausal symptoms or arthralgia/myalgia showed that overall survival and progression-free survival was significantly improved in patients reporting one of these symptoms versus those not reporting either symptom. In this analysis, the effect on overall survival and progression-free survival was irrespective of treatment.

Conclusions:
Our results suggest that the occurrence of menopausal symptoms or arthralgia/myalgia during treatment with tamoxifen or exemestane is associated with significantly improved overall survival.

P2-17-03
Incomplete Uptake and Diminished Adherence to Endocrine Therapy in Women with Hormone Receptor Positive Breast Cancer 4 Years from Diagnosis: An Australian Cohort Study.

Bell RJ, Fradin P, Schwarz M, Davis SR. Monash University, Melbourne, VIC, Australia; Alfred Health, Melbourne, VIC, Australia

Background: Tamoxifen(T) and the aromatase inhibitors (AIs) are associated with side effects which impair adherence to therapy. Our aim was to investigate the uptake of, and adherence to, endocrine therapy (ET) in a cohort of women with invasive breast cancer (BC) nearly 4 years post-diagnosis.

Materials and Methods: The BUPA Health Foundation Health and Well being After Breast Cancer study is a prospective questionnaire-based cohort study of 1684 women with their first episode of invasive BC recruited between 2004 and 2006 through the Victorian Cancer Registry (VCR), Australia. An enrolment questionnaire (EQ) was completed within 12 months of diagnosis, with questionnaires every 12 months thereafter (FQ1-FQ3). Tumor pathology was provided by the VCR. FQ3 was completed on average 3.8 years from diagnosis. Active disease included a new primary BC, local recurrence or metastatic disease.

Results: Of 1684 recruits, 1370 were HR+. At diagnosis, average age was 58 years and 50% were stage 1. The table shows the number of HR+ women retained in the analysis at each questionnaire, the average time of each questionnaire from diagnosis and the proportions of women at each questionnaire on any ET on T, an AI or on no ET.

<table>
<thead>
<tr>
<th>Questionnaire (n)</th>
<th>years from diagnosis (mean)</th>
<th>%ET</th>
<th>%T</th>
<th>%AI</th>
<th>% no ET</th>
</tr>
</thead>
<tbody>
<tr>
<td>EQ (1370)</td>
<td>0.8</td>
<td>65%</td>
<td>43%</td>
<td>22%</td>
<td>35%</td>
</tr>
<tr>
<td>FQ1 (1301)</td>
<td>1.8</td>
<td>87%</td>
<td>52%</td>
<td>35%</td>
<td>13%</td>
</tr>
<tr>
<td>FQ2 (1257)</td>
<td>2.8</td>
<td>84%</td>
<td>44%</td>
<td>40%</td>
<td>10%</td>
</tr>
<tr>
<td>FQ3 (1193)</td>
<td>3.8</td>
<td>82%</td>
<td>41%</td>
<td>41%</td>
<td>18%</td>
</tr>
</tbody>
</table>

Switching of ET from T to AI was predominantly a clinician decision. Cessation of T was patient initiated, mainly for vasomotor symptoms and vaginal bleeding. For the change from an AI to T, or cessation of an AI, the main reason was joint pain.

At FQ3, nearly 4 years from diagnosis, 215 (18%) women were not on any ET. Compared with women on ET at this time, these 215 women were more likely to have been stage 1 (rather than > stage 1) at diagnosis, have active disease by FQ3, be seeing an alternative therapist (all p<0.01) and were less likely to be seeing an oncologist (p<0.001).

Discussion:
By nearly 4 years from diagnosis, an increasing proportion of women with HR+ BC are not on ET, despite the evidence for the benefit of therapy for at least 5 years. Differences between ET users and non-users at this stage are not simply explained by previous users not continuing ET, with or without active disease. There is a small (8.2%) but important subgroup who appear to have never been on ET, which raises serious concerns.

P2-17-04
Induction of Tamoxifen Metabolism by Rifampicin: A Worrying Drug-Drug Interaction.

Binkhorst L, Loos WJ, de Jongh FE, Hamberg P, Ghobadi Moghaddam-Helmantel IM, Jager A, Sneyaeye C, Verweij J, van Gelder T, Mathijssen RH. Erasmus University Medical Center, Rotterdam, Netherlands; Erasmus University Medical Center - Daniel den Hoed Cancer Center, Rotterdam, Netherlands; Iakuzia Hospital, Rotterdam, Netherlands; Sint Franciscus Gasthuis, Rotterdam, Netherlands

Background: Tamoxifen (Tam) undergoes extensive biotransformation into several metabolites, including the highly active metabolite endoxifen. Differences in metabolism, influenced by both genetic and environmental factors, largely contribute to the inter-individual variability in endoxifen plasma concentrations, potentially affecting the efficacy of Tam. Conflicting results between CYP2D6 genotype/phenotype and endoxifen concentrations have been observed. A reason for this discrepancy may be that CYP3A4 may have a more crucial role in the formation of endoxifen than previously thought (de Graan et al, JCO, in press). Hypothesizing that induction of CYP3A4 and CYP2D6 could lead to increased endoxifen levels, a prospective

Cancer Res; 71(24 Suppl.) December 15, 2011 326s Cancer Research
study was activated evaluating the effects of enzyme induction by rifampicin (Rif) on the plasma pharmacokinetics (PK) of Tam and its metabolites.

Methods: A randomized cross-over study design was used, with each patient serving as her own control. Breast cancer patients on steady state Tam therapy (20 or 40 mg once daily) were included. Patients underwent two periods of plasma sampling covering 24 hrs each, once using Tam alone, and once after 15 days of oral Rif (600 mg daily) taken in combination with Tam. Patients were randomized for sampling sequence 1) Tam alone followed by Rif/ Tam versus 2) Rif/Tam followed by Tam alone. PK sampling in sequence 2 was performed after a 30-day wash-out period of Rif. Tam and its main metabolites ND-Tam, 4-OH-Tam and endoxifen were quantitated by a validated LC-MS/MS method. PK parameters, including area under the plasma-concentration time curve (AUC) and maximum concentration (Cmax) of all four compounds were calculated (WinNonLin program) and compared (with or without Rif) using a two-sided paired t-test. For safety reasons an interim-analysis was performed after 4 patients.

Results: The interim-analysis showed that concentrations of Tam and its metabolites were significantly decreased during Rif/Tam co-administration as compared to Tam administration alone (see table). Especially endoxifen exposure was decreased by a mean of 69%. Based on these data it was decided to stop further enrollment in this study.

Conclusions: Concentrations of Tam and its main metabolites decreased significantly after induction by rifampicin. Potentially contributing factors include further metabolism of Tam-metabolites into other inactive metabolites or conjugates (i.e. glucuronides) or a decreased oral availability of Tam. Further pharmacokinetic analyses, including the analysis of glucuronides and other metabolites, will be performed to better understand the mechanism behind these findings. Based upon these data, combining rifampicin with Tam should be avoided. Similar drug-drug interactions may exist between Tam and other strong CYP inducers, such as St John’s wort and phenytoin.

### Table

<table>
<thead>
<tr>
<th>Compound</th>
<th>Cmax (nM)</th>
<th>AUC (nM*h)</th>
<th>Ratio (with/without)</th>
<th>Sign.(P-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tam</td>
<td>329 (161)</td>
<td>4,632 (2,653)</td>
<td>0.24</td>
<td>0.02</td>
</tr>
<tr>
<td>ND-Tam</td>
<td>459 (227)</td>
<td>7,716 (4,100)</td>
<td>0.34</td>
<td>0.02</td>
</tr>
<tr>
<td>4-OH-Tam</td>
<td>4.8 (1.3)</td>
<td>1.8 (1.1)</td>
<td>0.28</td>
<td>0.01</td>
</tr>
<tr>
<td>Endoxifen</td>
<td>21.6 (10.3)</td>
<td>6.9 (2.5)</td>
<td>0.29</td>
<td>0.05</td>
</tr>
<tr>
<td>AUC</td>
<td>389 (133)</td>
<td>122 (57)</td>
<td>0.31</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Concentrations of Tam and its main metabolites were significantly decreased during Rif/Tam co-administration as compared to Tam administration alone (see table). Especially endoxifen exposure was decreased by a mean of 69% based on these data it was decided to stop further enrollment in this study.

### P2-17-05

**Antiprogestin Proellex Suppresses Proliferation of Aromatase Overexpressing and Letrozole Resistant T47D Breast Cancer Cells.**


**Background:** Aromatase is a cytochrome P450 CYP19A1 enzyme responsible for the conversion of C19 androgens to C18 estrogen. Aromatase overexpression leads to increased in local estrogen concentration in post menopausal women diagnosed for breast cancer. Aromatase inhibitors (AI) are used as one of the first line therapies for the treatment of ER+PR+ breast cancers. However, many patients acquire resistance to AI treatment. Therefore alternative approaches are being sought for patients with AI resistance. Previous reports suggest that upregulation of progesterone receptor can lead to enhanced expression of EGFR/ERK/MAPK involved in acquiring resistance. In this study, we evaluated the effect of a selective progesterone receptor modulator, CDB4124 (Proellex) with low glucocorticoid activity on aromatase overexpressing and Letrozole resistant T47D cells.

**Methods:** Aromatase overexpressing T47D (T47Darom) and respective control (T47Dcon) breast cancer cell lines were generated by stable transfection with plasmid containing CYP19A1 coding region, or empty vector respectively. Letrozole resistant cell line (T47DaromLR) was generated by incubating T47Darom for 75 weeks in the presence of 10µM Letrozole. Cell proliferation was determined by MTT or Crystal violet assays. Gene expressions were quantitated by qRT-PCR whereas proteins were identified by western blot analyses, flow cytometry and immunofluorescence staining. Aromatase activity was determined by estradiol ELISA. The effects of Proellex on anchorage independent growth were measured by soft agar colony formation. Statistical differences between the various groups were determined by Student’s ‘T’ test or ANOVA followed by Bonferroni’s post hoc test. Results: T47Darom and T47DaromLR cell lines had significantly higher aromatase expression (mRNA and protein) and as a result exhibited increased conversion of testosterone to estradiol as compared to T47Dcon. Both these cell lines showed enhanced growth in the presence of Testosterone. In T47Darom cells increased PR-B and EGFR expression as compared to T47Dcon cells was observed. Proellex, Letrozole and other known AI (Anastrozole, Exemestane) inhibited testosterone induced cell proliferation and anchor age independent growth of T47Darom cells. The inhibition of cell proliferation was significantly greater when cells were treated with Proellex in combination to other AIs. Proellex inhibited mRNA and protein levels of PR-B, reduced PRB/p300 complex formation in the nuclei and significantly reduced EGFR expression in T47Darom cells. Our results in the present study indicate that antiproliferative effect of Proellex may be due to PR-B / EGFR modulation in ER+PR+, aromatase overexpressing cells. Conclusion: Overall these results suggest that antiprogestin, Proellex could be developed as a possible treatment strategy for aromatase overexpressing ER+/PR+ breast cancer patients as well as for AI resistant breast cancer patients.

### P2-17-06

**Patterns of Bone Density Evaluation in a Community Population Treated with Aromatase Inhibitors.**

*Ligibel JA, O’Malley AJ, Fisher M, Daniel GW, Winer EP, Keating NL. Dana-Farber Cancer Institute, Boston, MA; Harvard Medical School, Boston, MA; HealthCore*

Aromatase inhibitors (AIs) lead to an increased risk of bone loss and fracture. Fracture rates in adjuvant AI studies have ranged from 2.3% to 11%, with higher rates seen in studies with longer duration of AI therapy and longer follow up. The American Society of Clinical Oncology recommends baseline bone density testing upon initiation of AI therapy and repeat testing every 1-2 years while on therapy. There are few data regarding the incorporation of these guidelines into clinical practice. We sought to evaluate patterns of bone density testing in a community-based cohort of breast cancer patients treated with AIs.

**Methods:** We obtained encounter and pharmacy data from the HealthCore Integrated Research Database, a fully integrated commercial payer dataset including patients enrolled in WellPoint insurance plans. We identified 9138 women aged ≥50 years who had at least 2 diagnosis codes for breast cancer between 2001 and 2007 (followed through 2008), and who filled at least 1 prescription for an AI. We identified
bone density testing using encounter data and used logistic regression to assess patient demographic and clinical characteristics associated with baseline bone density testing (any test from 6 months before through 6 months after the first prescription for AI). Among 2038 women who continued AI therapy for ≥2 years, we used logistic regression to assess factors associated with receipt of any bone density during the 2-year period.

**Results**

Overall, 41.6% of women underwent baseline bone density testing. Rates of bone density testing increased over time, from 26.6% in 2002 to 44.7% in 2008 (adjusted odds ratio [AOR] for 2008 vs. 2002=2.02, 95% confidence interval [CI]=1.53-2.68). Older women were less likely to undergo baseline bone density testing (AOR for women age ≥69 vs. 51-59=0.64, 95% CI=0.56-0.72). Women taking proton pump inhibitors, which have been linked to bone density loss, were also less likely to undergo baseline bone density testing (AOR=0.86, 95% CI=0.73-1.00). Women living in areas of lower education (p<.001), and areas with a higher proportion of blacks (p=0.02) or Hispanics (p<0.001), as well as women who were previously treated with tamoxifen (p=0.02), were all less likely to undergo baseline bone density. Women receiving bisphosphonates in the year before initiation of an AI were more likely to undergo baseline bone density testing (AOR=1.33, 95% CI=1.16-1.51).

Among women on AIs for at least 2 years, 59.9% of women underwent a bone density within 2 years of starting treatment. In adjusted analyses, earlier year of AI initiation (p<.001), living in areas with lower education levels (p<.001), and having been previously treated with tamoxifen (p<.001) were all associated with a decreased likelihood of undergoing bone density testing.

**Conclusions**

Despite the increased risk of fracture in women treated with AIs and guidelines recommending regular bone density evaluation, 58.4% of women starting an AI and 40.1% of women on long-term therapy did not undergo bone density evaluation in this community-based population. Older women, who might be especially vulnerable to bone loss and fracture, were less likely to undergo baseline bone density evaluation. More attention is needed to ensure that preventable fractures are avoided in patients taking AIs.

**P2-17-07**

**Construction of a Predictive Model of Probability of Ovarian Function Recovery in a Series of Premenopausal Breast Cancer Patients with Chemotherapy-Induced Amenorrhea Switched to an Aromatase Inhibitor (AI) after Adjuvant Tamoxifen.**

Perez-Fidalgo JA, Bermejo B, Pons V, Guzman C, Bosch A, Lluch A. Hospital Clínico Universitario, Valencia, Spain; Pfizer Spain, Alcobendas (Madrid, Spain).

Introduction: AI therapy is not recommended in breast cancer patients with conserved ovarian function as AI decreases estrogenic feedback leading to an increased of FSH and LH. In patients older than 40 and with prolonged amenorrhea, switching to an AI after tamoxifen therapy is a controversial approach but with an adequate follow-up for early detection of potential menopause renewal it might be feasible. Several factors have been identified in the literature as clinical defining variables of CIA while other factors have been associated with high probability of permanent CIA. Among clinical defining variables are time from last menses date (LMD) and low estradiol (E2) levels. Risk variables for permanent CIA are chemotherapy schedule administered and advanced age. We aimed to construct a predictive model to identify high risk of renewal of menses after switching to AI in premenopausal patients with CIA lasting at least 1 year.

Methods: Based on defining and prognostic variables of CIA, a predictive model of high probability of permanent amenorrhea was constructed by assigning a score as follows:

1) Time from LMD to switching date ≥3 years: 1 point, < 3 years: 0 points
2) E2 levels ≤20 ng/ml: 1 point, >20 ng/ml: 0 points
3) Age ≥45 years: 1 point, < or = 45: 0 points
4) Chemotherapy regimen administered: dose dense or high doses: 2 points, conventional doses regimen with anthracycline and taxanes: 1 point, conventional doses only anthracycline-based or other: 0 points.

Final score obtained from 0 to 5 points was classified in two groups:

- Low-probability of permanent amenorrhea: score 0-2, and high probability of permanent amenorrhea: score 3-5.

To validate this probability model we retrospectively analysed data from a prospective maintained database of early breast cancer patients, clinically premenopausal at diagnosis that were treated in our institution from May 2004 to December 2009. All patients had histologically confirmation of hormone-sensitive breast carcinoma at stage I-III.

Therapy included in all cases adjuvant or neoadjuvant chemotherapy and adjuvant tamoxifen and all patients referred a CIA lasting at least 1 year. Since 2006, in most patients E2 levels before switching were determined.

**Results:** Validation of probability model was performed in our series of 102 premenopausal patients of whom 9 recovered ovarian function (prevalence ratio: 0.088).

<table>
<thead>
<tr>
<th>Ovarian function recovery</th>
<th>No ovarian function recovery</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>score 0-2: low probability of permanent amenorrhea</td>
<td>9</td>
<td>40</td>
</tr>
<tr>
<td>score 3-5: high probability of permanent amenorrhea</td>
<td>6</td>
<td>55</td>
</tr>
<tr>
<td>total</td>
<td>9</td>
<td>95</td>
</tr>
</tbody>
</table>

Test sensibility of the model was 100% and specificity 56.99%. Positive predictive value was 18.4% and negative predictive value 100%. Positive likelihood ratio was 2.32 and negative likelihood ratio was 0.

**Conclusions:** Our model of probability of ovarian function recovery is a highly sensitive test that might become a useful tool to identify premenopausal patients with CIA that could be safely switched to AI after tamoxifen. Further validation in a prospective series including more accurate E2 monitoring and follow-up is warranted.

**P2-17-08**

**Prospective Study of Aromatase Inhibitor Induced Bone Loss and Lipid Levels in Early Postmenopausal (PM) Hormone Receptor Positive (HR+) Breast Cancer (BC) Patients Treated with Adjuvant Letrozole Extended beyond 5 Years (yrs).**


Introduction/Aim: Adjuvant treatment with aromatase inhibitors (AI) is associated with accelerated bone loss and increase in lipid levels. The effectiveness and tolerance of extended treatment with AI beyond 5 years is currently under investigation. In the SOLE study, five years of continuous (C) extended letrozole is compared to interrupted therapy (9 months on, 3 months off letrozole) after 5 yrs of tamoxifen, AI or sequential hormonal therapy. We have evaluated the bone mineral density and lipid levels during the extended use of letrozole beyond 5 yrs in a cohort of patients included in the SOLE study.

Patients and methods: Postmenopausal women with HR+ BC, receiving extended continuous (C) or intermittent (I) letrozole for 5 yrs after 5 yrs of tamoxifen, 5 yrs of an AI, or switch therapy within...
Background: Invasive lobular carcinoma (ILC) represents the second most common BC subtype and is often characterized as hormone responsive to trastuzumab in patients with advanced HER2+ lobular breast carcinoma. In addition, we sought to describe the pattern of hormone receptor positivity in the subsets of ILC and invasive ductal carcinoma (IDC).


Metzger O, Procter M, de Azambuja E, Viale G, Leyland-Jones B, Dowsett M, Gelber R, Gresko E, Loi S, Sotiriou C, Piccart M. Institut Jules Bordet, Université Libre de Bruxelles, Brussels, Belgium; Frontier Science (Scotland) Ltd, Kincraig, Kingussie, United Kingdom; European Institute of Oncology, Milan, Italy; Emory University, Atlanta; Royal Marsden Hospital and Institute of Cancer Research, London, United Kingdom; Dana-Farber Cancer Institute, Boston; Roche, Basel, Switzerland.

Background: Invasive lobular carcinoma (ILC) represents the second most common BC subtype and is often characterized as hormone receptor positive and HER2-negative. However, a small subset of ILC is found to be HER2+. Isolated case reports have demonstrated high responsiveness to trastuzumab in patients with advanced HER2+ ILC. The HERA trial comprises 1 or 2 years of trastuzumab treatment with observation and 1 versus 2 years of trastuzumab treatment after standard chemotherapy in women with HER2+ breast cancer. We sought to evaluate the incidence of HER2+ ILC and the magnitude of trastuzumab benefit in HER2+ ILC in the context of the HERA trial. In addition, we sought to describe the pattern of hormone receptor positivity in the subsets of ILC and invasive ductal carcinoma (IDC) as well as the pattern of hormone receptor positivity among ILCs.

Methods: The database used in the analysis had a clinical cut-off date of 9th June 2008 and 4-year median follow-up (Gianni et al., 2011). Patients randomized to the 1-year trastuzumab and observation group were included in the preliminary study. 18% of ILC-users discontinued their treatment due to musculoskeletal symptoms compared with none of the tamoxifen-users. 62% of patients on AI and 35% of tamoxifen patients complained of new or worsened joint pain. Table 1 shows the proportion of patients that attribute their complaints to their endocrine therapy. Grip strength significantly decreased over time (p<0.03), with patients under AI treatment having a larger loss of grip strength than patients under tamoxifen treatment (p=0.04).

We confirm our previously reported results on the shape of the curve “BMI AI-induced loss of grip strength”. Years past menopause and age showed a significant effect on grip strength (p<0.001 and p=0.0007, respectively), whereas no significant relationship was found between WHR with grip strength or joint pain. Detailed results will be presented.

Conclusion Our preliminary results confirm that a majority of patients treated with an AI experience musculoskeletal problems, which are considered due to the therapy by most patients. Grip strength decreased over time, with a significantly larger loss of grip strength in the AI-users compared with the tamoxifen-users. AI-induced loss of grip strength and baseline BMI showed an inverse relationship, although differences between quartiles were small. Further results should be awaited as this is only a preliminary analysis.

Table 1 Percentage of patients assigning their endocrine therapy as causal factor for the side effects

<table>
<thead>
<tr>
<th>Side Effect</th>
<th>Aromatase Inhibitor</th>
<th>Tamoxifen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Joint pain</td>
<td>71%</td>
<td>17%</td>
</tr>
<tr>
<td>Joint stiffness</td>
<td>75%</td>
<td>50%</td>
</tr>
<tr>
<td>Hot flashes</td>
<td>85%</td>
<td>82%</td>
</tr>
<tr>
<td>Muscle aches</td>
<td>60%</td>
<td>7%</td>
</tr>
<tr>
<td>Numbness</td>
<td>62%</td>
<td>9%</td>
</tr>
<tr>
<td>Cognition problems</td>
<td>38%</td>
<td>38%</td>
</tr>
<tr>
<td>Sexual problems</td>
<td>60%</td>
<td>65%</td>
</tr>
</tbody>
</table>

Conclusion: This is the first prospective BMD and lipid follow-up study during adjuvant letrozole extended beyond 5 years in PM early breast cancer patients. After the first year of extended intake of continuous letrozole, patients will be presented. After one year no differential effect on cholesterol levels has been observed.

P2-17-09

A Prospective Assessment of Loss of Grip Strength by Baseline BMI in Breast Cancer Patients Receiving Adjuvant Aromatase Inhibitors or Tamoxifen.


Background: The 3rd generation aromatase inhibitors (AIs) induce or enhance musculoskeletal problems. Underlying mechanisms are probably multiple, but remain unknown. We have previously reported that loss of grip strength together with tenosynovial abnormalities are more important in AI- than in tamoxifen-users (Morales et al, JCO 2008) and that musculoskeletal changes in AI-users are more pronounced in women with extremes in baseline BMI (Lintharms et al, Ann Oncol 2011) We here report preliminary results from a larger population and plan to validate findings in patients from University of Michigan.

Patients and methods: In this prospective observational study, postmenopausal early breast cancer patients scheduled to start adjuvant hormonal therapy with any of the third generation AIs or tamoxifen were recruited. After providing informed consent, a modified sphygmomanometer was used to assess and a rheumatological questionnaire was completed at each visit. Results: Ninety-four (79 AI; 15 tamoxifen) of the planned 200 patients were included in this preliminary study. 18% of AI-users discontinued their treatment due to musculoskeletal symptoms compared with none of the tamoxifen-users. 62% of patients on AI and 35% of tamoxifen patients complained of new or worsened joint pain. Table 1 shows the proportion of patients that attribute their complaints to their endocrine therapy. Grip strength significantly decreased over time (p<0.03), with patients under AI treatment having a larger loss of grip strength than patients under tamoxifen treatment (p=0.04).

We confirm our previously reported results on the shape of the curve “BMI AI-induced loss of grip strength”. Years past menopause and age showed a significant effect on grip strength (p<0.001 and p=0.0007, respectively), whereas no significant relationship was found between WHR with grip strength or joint pain. Detailed results will be presented.

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<td>50%</td>
</tr>
<tr>
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<td>85%</td>
<td>82%</td>
</tr>
<tr>
<td>Muscle aches</td>
<td>60%</td>
<td>7%</td>
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<tr>
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Conclusion: This is the first prospective BMD and lipid follow-up study during adjuvant letrozole extended beyond 5 years in PM early breast cancer patients. After the first year of extended intake of continuous or intermittent letrozole a possible difference in bone mineral density between the two arms is emerging, but the two groups are too small to make final conclusions. An updated longer follow-up on more patients will be presented. After one year no differential effect on cholesterol levels has been observed.

P2-18-01

The Magnitude of Trastuzumab Benefit in HER2-Positive (HER2+) Lobular Breast Carcinoma (BC): Results of a HERA Trial Sub-Group Analysis.

Metzger O, Procter M, de Azambuja E, Viale G, Leyland-Jones B, Dowsett M, Gelber R, Gresko E, Loi S, Sotiriou C, Piccart M. Institut Jules Bordet, Université Libre de Bruxelles, Brussels, Belgium; Frontier Science (Scotland) Ltd, Kincraig, Kingussie, United Kingdom; European Institute of Oncology, Milan, Italy; Emory University, Atlanta; Royal Marsden Hospital and Institute of Cancer Research, London, United Kingdom; Dana-Farber Cancer Institute, Boston; Roche, Basel, Switzerland.

Background: Invasive lobular carcinoma (ILC) represents the second most common BC subtype and is often characterized as hormone receptor positive and HER2-negative. However, a small subset of ILC is found to be HER2+. Isolated case reports have demonstrated high responsiveness to trastuzumab in patients with advanced HER2+ ILC. The HERA trial comprises 1 or 2 years of trastuzumab treatment with observation and 1 versus 2 years of trastuzumab treatment after standard chemotherapy in women with HER2+ breast cancer. We sought to evaluate the incidence of HER2+ ILC and the magnitude of trastuzumab benefit in HER2+ ILC in the context of the HERA trial. In addition, we sought to describe the pattern of hormone receptor positivity in the subsets of ILC and invasive ductal carcinoma (IDC) as well as the pattern of hormone receptor positivity among ILCs.

Methods: The database used in the analysis had a clinical cut-off date of 9th June 2008 and 4-year median follow-up (Gianni et al., 2011). Patients randomized to the 1-year trastuzumab and observation
arms were included in the present analysis. Central assessment of hormone receptor status was considered. Histological BC subtype was assessed locally.

Results: Of the 1703 women randomized to one-year of trastuzumab and 1698 to observation, 5.5% (n=187) and 94.5% (n=3213) were diagnosed as HER2+ ILC and IDC, respectively. Central hormone receptor status was available in 88.3% (n = 2838) of IDC and 86.1% (n=161) of ILC. ER and/or PR positivity was more common in IDC than ILC (63.4% [102/161] vs. 46.3% [1314/2838]; p<0.001). Allred scores for ER are shown in the table below. The DFS hazard ratios comparing one year of trastuzumab versus observation were 0.63 (95% CI 0.34-1.14) for ILC and 0.77 (95% CI 0.67-0.89) for IDC. There was no evidence of an interaction between histological subtype and trastuzumab benefit (interaction [subtype lobular and subtype ductal and not lobular] p=0.49).

<table>
<thead>
<tr>
<th>Summary of Central Assessment of Hormone Receptor Status by Type of Primary Breast Cancer</th>
<th>Lobular (HER2+)</th>
<th>Ductal (HER2+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hormone receptor status</td>
<td>N = 187</td>
<td>N = 3213</td>
</tr>
<tr>
<td>Positive</td>
<td>102 (55.4%)</td>
<td>1314 (40.9%)</td>
</tr>
<tr>
<td>Negative</td>
<td>75 (40.1%)</td>
<td>1909 (59.1%)</td>
</tr>
<tr>
<td>Estrogen receptor status</td>
<td>N = 160</td>
<td>N = 2835</td>
</tr>
<tr>
<td>Positive</td>
<td>92 (45.0%)</td>
<td>1777 (62.7%)</td>
</tr>
<tr>
<td>Negative</td>
<td>68 (35.0%)</td>
<td>1058 (37.3%)</td>
</tr>
</tbody>
</table>

Conclusion: HER2+ ILC accounts for 5.5% of patients included in a large population of over 3,000 HER2+ BC. While only a limited number of patients with ILC was enrolled, this analysis suggests an increased ER positivity in ILC compared to IDC. There was no suggestion that patients with HER2+ ILC derived a different magnitude of benefit from adjuvant trastuzumab when compared to the HER2+ IDC cohort. The lack of central pathology review for BC subtype assessment is a caveat of our study. Future research in the field of ILC and particularly in the HER2+ subset should be encouraged.

P2-18-02
Cardiac Outcomes of Patients on Adjuvant Weekly Paclitaxel (T) and Trastuzumab (H) for Node Negative, HER2 Positive Breast Cancer (BCA).

Dang C, Tolaney S, Najita J, Gelman R, Yardley D, Marcom K, Albain K, Rugo H, Miller K, Ellis M, Shapira I, Wolff A, Carey L, Vahdat L, Burdette-Radous S, Budd T, Krop I, Burstein H, Hudis C, Winer E. Memorial Sloan-Kettering Cancer Center; Dana-Farber Cancer Institute; Tennessee Oncology and Sarah Cannon Research Institute; Duke University; Loyola University Medical Center; University of California-San Francisco Comprehensive Cancer Center; Indiana University; Washington University; North Shore-Long Island Jewish Medical Center; Johns Hopkins University; University of North Carolina; Weill Cornell Medical College; University of Vermont; Cleveland Clinic Taussig Cancer Institute Case

Background: Twenty-five percent of node-negative HER2-positive BCA have a higher risk of recurrence than those with node-negative HER2-negative disease. Several randomized trials have shown the benefit of chemotherapy (most with an anthracycline) and trastuzumab (H) for pts with node-negative and high-risk node-negative BCA. However, the benefit of chemotherapy and H needs to be further explored in the node-negative group. Due to the 2-4% risk of symptomatic congestive heart failure (CHF) with an anthracycline-based treatment (Rx) followed by H, we set out to conduct a study of a taxane-based Rx with H in a node-negative population.

Design: This is a single arm, multicenter, phase II study of paclitaxel (T) (80 mg/m²) and trastuzumab (H) x 12 weekly (w) (4 mg/kg load \(-2 mg/kg\) \(\rightarrow\) H x 52 w (2 mg/kg weekly or 6 mg/kg q 3 w). Pts with HER2-positive BCA (IHC 3+ or FISH amplified at \(\geq 2.0\)) with negative nodes (micrometastasis later allowed) and with a tumor size \(\leq 3\) cm were enrolled. The primary endpoint is disease-free survival. Secondary endpoints include the incidence of G 3/4 left ventricular systolic dysfunction or congestive heart failure (CHF). Pts had serial left ventricular ejection fraction (LVEF) monitored at baseline (BSLN), and at month (mo) 3, mo 6, and mo 12 w/ a multi-gated acquisition scan or echocardiogram. H was held for significant asymmetrical (Asx) LVEF \(\downarrow\) (10-15% \(\downarrow\) from BSLN and < lower limit of normal or \(\geq 16%\) \(\downarrow\) from BSLN), and H was stopped for CHF.

Results: From 10-9-2007 to 9-3-2010, 406 pts were enrolled. The median (med) age was 55 years (range 23-84 years); 118/406 (29%) had hypertension and 30/406 (7%) had diabetes. As of 6-1-2011, 307 are reported as off Rx of which 261 have completed protocol therapy; 99 remain on therapy. To date, 100%, 94%, 84%, and 83% of pts had LVEF monitoring at BSLN, mo 3, mo 6, and mo 12. The med LVEF at BSLN was 65% (range 50%-81%), at mo 3 was 64% (range 45%-81%), at mo 6 was 64% (range 45%-81%), and at mo 12 was 65% (range 37%-90%). Two pts had CHF; 1 pt had CHF at mo 11 (LVEF was 55% at BSLN and 37% w/ CHF event) and 1 pt had CHF at mo 6 (LVEF was 66% at BSLN and 49% w/ CHF event). To date, 13 pts had H held for significant Asx LVEF \(\downarrow\); details on those who had appropriate LVEF recovery and had H restarted will be provided. All patients will have completed 12 mo of Rx by October 2011, and an accurate report of the incidence of CHF will be available.

Conclusion: This represents the cardiac report of pts receiving TH as adjuvant Rx for node-negative (micrometastasis allowed) HER2 positive BCA. Both the CHF events and number of pts w/ H hold due to significant Asx LVEF \(\downarrow\) appear low. Overall, the serial med LVEFs remain stable throughout the 12 mo of Rx. Final data on all 406 pts will be available in December 2011.

P2-18-03
Systemic Adjuvant Treatment of T1a and T1b N0M0 HER2+ Breast Carcinomas; an AERIO/UNICANCER Study.

Peron J, Vano Y, Frenel J-S, Wassermann J, Albigez L, Rodrigues MJ, Coutte PH, Centre Léon Bérard, Lyon, France; Centre Antoine Lacassagne, Nice; Institut de Cancérologie de l'Ouest, Centre René Gauducheau, Nantes; Institut Curie, Centre René Huguenin, Saint-Cloud; Institut Gustave Roussy, Villejuif; Institut Curie, Paris

Background: Trials have shown benefit of adjuvant trastuzumab (TZM) for node-negative (N-) or T1c+ HER2-positive breast carcinomas. Methods: Retrospective series in 6 french cancer centers from 2000 to 2010 of T1abN0M0 HER2 positive breast carcinomas. Multifocal tumors were excluded.

Results: Two hundred five N- cases were retrieved. Median size was 8 mm (range, 2 to 10 mm), 51 were T1a (25%), 152 tumors were T1b; 120 tumors (59 %) exhibited significant hormonal receptors (HR) expression. All patients had surgery, 65 % (n= 133) had a local irradiation. Ninety percent of HR+ patients (108/120) received hormonal therapy: 77 received aromatase inhibitors (AI) upfront or sequential; 23 received tamoxifen (TAM) alone 13 received LHRH agonists alone or in combination with TAM or AI. Forty-nine percent (n=101) had chemotherapy (CT) (Anthracycline alone for 41 cases, taxane alone for 31 cases, sequential A/T for 28 cases and concurrent for 1 case), associated with TZM in 90 cases. TZM was administered without chemotherapy in 3 cases. Decision of adjuvant CT and/or
TZM was associated with (all p<0.05) HR-negative status, Elston-Ellis grade 2/3 and high mitotic index (MI) while patients with HR+/low MI tumors were rarely treated (p=0.01). With a 41 months median follow-up, there was a statistically significant difference in invasive recurrences between TZM treated patients and others (log-rank test p=0.04). Twelve of the 112 patients treated without TZM (11%) had a recurrent invasive disease including 6 metastatic cases and 3 fatal events. There was one invasive recurrence in TZM group. There were as much recurrences in T1a as in T1b tumors. Three of the 12 recurrences (25%) in the group without TZM occurred in T1a cases.

Conclusion: TZM was associated with (all p<0.05) HR-negative status, Elston-Ellis grade 2/3 and high mitotic index (MI) while patients with HR+/low MI tumors were rarely treated (p=0.01). With a 41 months median follow-up, there was a statistically significant difference in invasive recurrences between TZM treated patients and others (log-rank test p=0.04). Twelve of the 112 patients treated without TZM (11%) had a recurrent invasive disease including 6 metastatic cases and 3 fatal events. There was one invasive recurrence in TZM group. There were as much recurrences in T1a as in T1b tumors. Three of the 12 recurrences (25%) in the group without TZM occurred in T1a cases.

P2-18-04
Pilot Evaluation of Bevacizumab (Bev) in Combination with Docetaxel (T) and Cyclophosphamide (C) as Adjuvant Treatment (AdjRx) for Patients (pts) with Early Stage (ES) Breast Cancer (BrCa).

Background: The combination of Bev + chemotherapy (CRx) has been shown to produce superior response rates and progression free survival compared to CRx alone, providing a rationale for the study of Bev with AdjCRx for pts with ESBrCa. As Bev can cause hypertension (HTN) and may increase the risk of cardiac failure, there is a rationale for studying a standard non-anthracycline (Anth) AdjCRx with Bev, e.g. TC (docetaxel-cyclophosphamide). We performed a pilot phase II study to evaluate the feasibility and toxicity of TC+Bev in pts with ESBrCa in preparation for participation in a random assignment trial. We have previously reported preliminary toxicity data. Methods: Eligibility criteria included: ESBrCa which was HER-2 normal, node+ or, N- and primary tumour (T) >2 cm and receptor negative, or T>3 cm, normal left ventricle ejection fraction (LVEF), no active/uncontrolled cardiovascular disease, normal organ and marrow function. Treatment consisted of four 3-weekly cycles of docetaxel 75 mg/m2 together with cyclophosphamide 600 mg/m2. Patients commenced Bev 15 mg/kg i.v. on day 1, and then every 3 weeks to a total of 18 cycles of treatments. Pts were monitored clinically, with echocardiograms and with serial estimations of BNP and troponin.

Results: A total of 106 female pts were accrued in 9 ICORG sites between 11/2008 and 7/2010. Ages ranged from 25-75 (median 52). On 20/06/2011, 105 pts have completed study Rx, 1 will finish 7/2011. A total of 36 serious adverse events (SAEs) have been reported so far, 33 involving hospital admission, 3 serious for other reasons. In 25 (24%) pts study Rx was discontinued due to: HTN-9, intestinal perforation-2, consent withdrawl-7, infection-2, proteinuria-1, anaphylaxis-1, cancer relapse-1, arthralgia-1, anal fistula-1. The two perforations occurred at cycles 1 and 16 of Bev respectively. Neither pt with perforation had history of prior abdominal surgery. The median number of cycles achieved by the discontinued pts was 9. HTN of any grade occurred in 49 out of 103 (48%) pts who had no HTN at baseline (BL) and 42 of them required Rx. Among pts who experienced HTN on study Rx and completed Bev, 34 (81%) were still on anti-hypertensive 4 weeks after last infusion of Bev. Forty-one (39%) pts had LVEF drop >10% from BL during study Rx. In 8 (7.5%) pts LVEF declined below 50%, 6 are documented to have recovered to normal, 2 had no further LVEF measurements (1 declined, 1 unknown reason). No episodes of CCF were reported. Troponin and BNP levels were normal in all 57 pts with serial measurements. Forty-four pts required treatment for neutropenia-related infection or for abscess/fistula. Conclusions: In this study Bev overall toxicity in ESBrCa pts was similar to that reported for pts with MBC, and Bev discontinuation due to toxicity was relatively frequent. Although no pt developed CCF 7.5% of decline in LVEF<50% was observed. Intestinal perforation can occur in ESBrCa pts in absence of prior intestinal surgery and in the post-CRx phase of Bev. Pts receiving Bev with non-Anth AdjCRx require careful monitoring for toxicity.

P2-18-05
Cardiotoxicity Risks of Adjuvant Trastuzumab in Asian Breast Cancer Patients.
Shih V, Chan A, Sim MH, Teo C, Chen W, Wong ZW. National Cancer Centre, Singapore; National University of Singapore, Singapore

Background: Adjuvant trastuzumab (T)-based chemotherapy reduces relapse and improves overall survival in early breast cancer. However, T-associated cardiotoxicity potentially limits its use. Our study aims to report the incidence, severity and reversibility of cardiotoxicity amongst Asian breast cancer patients.

Methods: This is a retrospective review of patients who have received adjuvant T from June 2005 to March 2011 at our centre. CT was defined as a drop in left ventricular ejection fraction (LVEF) to less than 50% and / or reduction of ≥ 10% of baseline. Cardiovascular (CVS) risk factors were defined as having at least one of the following factors: family or previous history of CAD, hypertension, diabetes mellitus, hyperlipidemia and smoking. One-way repeated measures ANOVA was used to evaluate the mean LVEF change and Chi-square test to evaluate the association of demographics and CT.

Results: A total of 314 female patients were reviewed. CT was reported in 124 (39.5%), of whom 96 had asymptomatic decline in LVEF and 28 were symptomatic. T was withheld (n=53) due to asymptomatic decline in LVEF (n=40), symptomatic heart failure (n=3) and both (n=10). Forty-three patients with resolution of CHF (n=11) or LVEF recovery (n=32) were rechallenged. Cardiotoxicity recurred in 14 - asymptomatic decline in LVEF (n=10), recurrent CHF (n=1) and both (n=3). Overall, there were no cardiac-related deaths. Factors that predicted for CT included low normal pre-trastuzumab LVEF (<60%) (p=0.018). Three-monthly LVEF showed statistically significant decline against baseline (p=0.003). As expected, the decline in LVEF demonstrated significant interaction with CT groups and non CT group (p<0.001).
Conclusions: A higher incidence of CT (39.5%) was observed among Asian breast cancer patients compared to previously reported in Caucasians (24%). Although 77.4% of patients presented with asymptomatic decline in LVEF, approximately one-third of patients experienced recurrent CT upon rechallenge.

P2-18-06
Conventional Trastuzumab Is an Antagonist of Natural Killer Cells: Making the Case for Fucose-Depleted Trastuzumab.

Listinsky JJ, Siegel GP, Listinsky CM. University of Alabama at Birmingham, Birmingham, AL; Case Western Reserve University, Cleveland, OH

The discovery of the HER2 receptor and later the development of trastuzumab (an anti-HER2 antibody) were major advances leading toward the treatment of a significant proportion of human breast cancers. Trastuzumab (Herceptin®) is a humanized monoclonal antibody. Trastuzumab's FAB portion binds to the HER2 extracellular domain, interrupting HER2 signaling. The FC components of the antibody. Trastuzumab's Fc portion binds to the FC receptors located on effector cells. The superior ADCC effect offers several potential advantages over conventional trastuzumab. Such advantages in clinical use, we postulate, include (1) treatment of patients with trastuzumab-resistant tumors, (2) treatment of patients with equivocal or low expression of HER2, and (3) decreased cost of treatment.

P2-19-01
Impact of Zoledronic Acid on Fractures, Bone Mineral Density and Bone Remodeling in the AZURE Trial (BIG 01-04).

Coleman R, Woodward E, Turner L, Marshall H, Collins M, Dodwell D, Davies C, Bell R, Cameron D, Brown J. University of Sheffield, Sheffield, United Kingdom; University of Leeds, Leeds, United Kingdom; Andrew Love Cancer Centre, Geelong, Victoria, Australia; Western General Hospital, Edinburgh, United Kingdom

Background: The AZURE trial was designed to determine whether the addition of zoledronic acid (ZOL) to standard adjuvant therapy improves disease free survival (DFS) in patients (pts) with stage II/III breast cancer. Although in a recent analysis after 752 events and a median follow-up of 59 months no difference in overall DFS was seen between ZOL and control pts, significant benefits were seen in women >5years post menopause. Here, we report the impact of ZOL on fractures in the main study, and on bone mineral density (BMD) and bone remodeling evaluated within a sub-study.

Materials and methods: 3360 pts were randomized to receive (neo) adjuvant chemotherapy (CT) and/or endocrine therapy with (n=1681) or without (n=1678) up to 19 doses of ZOL 4mg over 5 years. Pts with osteoporosis and those using bisphosphonates, either at baseline or in the previous year, were excluded from study entry. BMD was assessed in 228 patients within a sub-study; 40 of these also underwent quantitative bone scanning (QBS), a novel imaging technique that yields values for \(^{99m}\)Tc-MDP/HMDP whole skeleton plasma clearance (\(K_{wss}\)) as a surrogate for the rate of bone remodeling and enables assessment of specific regions of interest within the skeleton.

Results: Fractures occurred in 152 (4.5%) pts; 60 (3.6%) ZOL pts compared with 92 (5.5%) control (CTRL) pts (difference -0.9%; 95%CI -1.6%, -0.2%). For pts with a DFS event, the majority of fractures in the ZOL group and 68 (61.8%) in the CTRL group (difference -0.4%; 95%CI -10.2%, -3.7%). For pts with a DFS event, the majority of fractures occurred after a skeletal recurrence; 87.5% (7 of 8) ZOL pts and 68 (61.8%) in the CTRL group (difference -1.9%; 95%CI -3.3%, -0.5%). The difference in fracture incidence appeared early and persisted throughout the follow-up period. Fifty-six (86.2%) of the fractures in the ZOL group and 68 (61.8%) in the CTRL group occurred in the absence of, or prior to a DFS event. The fracture rates before disease recurrence were similar at 3.0% (51 pts) in the ZOL group and 3.4% (57 pts) in the CTRL group (difference -0.4%; 95%CI -1.6%, 0.8%). In contrast, 2.1% (8 ZOL pts) and 9.1% (34 CTRL pts) experienced a fracture after a DFS event (difference -6.9%; 95%CI -10.2%, -3.7%). For pts with a DFS event, the majority of fractures occurred after a skeletal recurrence: 87.5% (7 of 8) ZOL and 88.2% (30 of 34) CTRL pts. BMD Z-scores at completion of protocol treatment were higher in patients treated with ZOL. \(K_{wss}\) was lower in ZOL patients (26.5 vs 29.8 ml min\(^{-1}\) using \(^{99m}\)Tc-MDP and 34.3 vs 39.3 ml min\(^{-1}\) with \(^{99m}\)Tc-HMDP). Expressed as a percentage of whole skeleton clearance, regional values in the mandible were similar 1.15% (ZOL) vs. 1.22% (CTRL).

Conclusions: Adjuvant ZOL given in the schedule studied in AZURE reduced the fracture rate in patients who developed recurrence of
breast cancer. BMD was higher and the rate of bone remodeling less in patients treated with ZOL. Further follow-up will determine the duration and clinical relevance of these effects on bone health.

**P2-19-02**  
**Multidisciplinary Treatment of Pregnancy-Associated Breast Cancer.**  
Meisel JL, Economy KE, Zabicki-Calivillo K, Gelber S, Kereakoglow S, Winer EP, Partridge AH, Mayer EL. Brigham and Women’s Hospital, Boston, MA; Dana-Farber Cancer Institute, Boston, MA  

**Background**  
Breast cancer complicates pregnancy in a significant minority of younger breast cancer (BC) patients (pts). Application of standard treatment algorithms is limited by the lack of randomized data to support safety and efficacy. A multidisciplinary approach attempts to maximize treatment efficacy for a pt while minimizing fetal toxicity. We sought to describe contemporary multidisciplinary BC treatment in an academic setting and explore early maternal and fetal outcomes.  

**Methods**  
A search of the Dana-Farber/Harvard Cancer Center clinical database was performed to find BC pts self-identified as pregnant at presentation with >2 visits at our institution. Information available within the database along with complementary chart review provided sociodemographic, disease, staging, pregnancy and treatment information as well as short-term maternal and fetal outcomes.  

**Results**  
55 pts diagnosed between 1996-2011 were identified. The median age at diagnosis was 34 years. 25.5% were stage I, 49.1% stage II, 20% stage III, and 5.4% stage IV. 63.6% had hormone receptor positive disease, 36.3% HER2 positive, and 18.1% triple negative. 71% underwent testing for germline BRCA 1/2 mutations, with 9% of all pts testing positive. 29% were diagnosed in the first trimester (T1), 29% in T2, and 42% in T3. 89% underwent ultrasound imaging for staging, 49% X-ray imaging, 16.3% MRI, and 0% CT. 67% underwent surgery during pregnancy: 43.2% mastectomy, 48.6% lumpectomy, and 8.1% lumpectomy with subsequent mastectomy during pregnancy. 18.9% underwent surgery in T1, 45.9% in T2, and 37.8% in T3. 27.2% underwent sentinel lymph node biopsy. 51% received chemotherapy (C) during pregnancy: of those, 100% received anthracycline/cyclophosphamide (2-4 cycles), 11% paclitaxel, and 0% trastuzumab. 28.5% received C on a dose-dense schedule, with 25% supported by growth factors (14.2% filgrastim, 10.8% pegfilgrastim). 28.5% received neoadjuvant C. C was initiated during T1 for 0%, T2 for 64.3%, and T3 for 35.7%. Two pts terminated pregnancy in T1, one spontaneously miscarried at 12 weeks (wks), and two are currently in the third trimester of pregnancy; therefore, a total of 50 pts had delivered at the time of this analysis. The median time of delivery was 36 wks. 50% delivered prior to 37 wks and were considered preterm; of those, 76% were inductions or Caesarian sections planned to facilitate cancer therapy. Only 12% delivered prior to 34 wks. For the 25 infants for whom Pappar scores were available, 76% had scores of ≥ 8 at delivery, and 100% had scores of ≥ 8 at 5 minutes. For the 25 infants for whom birth weights were available, the median birth weight was 6lbs 1oz. Only 4 were less than 5lbs at the time of delivery. A total of 4 fetal abnormalities were noted: cleft palate (2), club foot (1), and ventricular septal defect (1).  

**Conclusions**  
Within a multidisciplinary academic center, treatment of pregnancy-associated BC using contemporary treatment algorithms, including taxane chemotherapy, growth factor support, and sentinel lymph node biopsy, has been pursued without significant adverse effect on fetal outcomes when compared to other published series. A considerable number of preterm deliveries have been observed. Further data collection is ongoing for confirmation of initial observations.

**P2-19-03**  
**Influence of Zoledronic Acid on BMD in Premenopausal Women with Breast Cancer and Neoadjuvant or Adjuvant Chemotherapy and/or Endocrine Treatment – The ProBone Studies.**  
Hadiji P, Kaouka A, Bauer T, Kaldner M, Albert U, Birkholz K, Baier M, Math M, Ziller M. Philipps-University of Marburg, Marburg, Germany; Novartis Pharma GmbH, Nuernberg, Germany  

**Background**  
Based on baseline bone mineral density (BMD), adjuvant chemotherapy or endocrine therapy for early breast cancer patients can lead to substantially increased fracture risk. A significant decrease of BMD >10% after 2 years of chemotherapy (CT) and/or endocrine therapy (ET) has been reported. In recent studies, zoledronic acid (ZOL) produced an increase in BMD in premenopausal and postmenopausal patients with breast cancer (ABCGS-12, Z-FAST, ZO-FAST, etc). In addition, a significant increase in disease-free survival (DFS) with ZOL vs no ZOL was observed in most of these studies.  

**Methods**  
The aim of 2 single-center, placebo-controlled, randomized studies—Probone I and Probone II—was to investigate the effect of adjuvant treatment with ZOL on BMD in premenopausal women with early breast cancer treated with CT and/or ET. Patients with hormone-receptor–negative (HR-) breast cancer (Probone I) were treated with (neo)adjuvant CT; patients with hormone-receptor–positive (HR+) breast cancer (Probone II) were treated with ET alone or in combination with (neo)adjuvant CT. Randomized patients received ZOL 4 mg or placebo IV every 3 months for 24 months. The primary objective was the change in BMD at the lumbar spine between baseline and month 24 (measured by dual-energy X-ray absorptiometry [DXA]). Secondary objectives included DFS; BMD at total hip, femur, and os calcis; quantitative ultrasonometry (QUS) at os calcis and phalanges; markers of bone turnover (C-telopeptide of type I collagen [CTX] and N-terminal propeptide of type I procollagen [P1NP]); endocrine hormones (follicle-stimulating hormone [FSH], estradiol, testosterone, sex hormone-binding globulin [SHBG], parathyroid hormone [PTH], vitamin D, anti-Müllarian hormone [AMH], inhibin A/B, etc); pathologic fractures; and safety and tolerability.  

**Results**  
70 HR+ and 11 HR- breast cancer patients have been enrolled into the studies. The last patient will have been treated for 24 months by the end of June 2011.  

**Conclusions**  
The effects of ZOL on lumbar spine BMD at 24 months and secondary endpoints will be presented at the meeting.

**P2-19-04**  
**Changes in Adjuvant Treatment of Early Breast Cancer in Italy between 2000 and 2008. The NEMESI Study Versus the NORA Study.**  
Mustacchi G, Cazzaniga ME, Pronzato P, Clavarezza M, Del Mastro L, Venturini M, Amadori D, Bidoli P, Boni C, Caruso M, RicCARDI F, DonD I. Università, Trieste, Italy; Ospedale San Gerardo, Monza, Italy; IRCCS Istituto Nazionale Ricerche sul Cancro, Genova, Italy; Ospedale Sacro Cuore Don Calabria, Negrar (VR), Italy; I.R.S.T., Meldola (Forlì), Italy; Azienda Ospedaliera S Maria Nuova, Reggio Emilia, Italy; Centro Oncologico Catanesi, HumanaItis, Catania, Italy; Ospedale Cardarelli, Napoli, Italy; Sanofi Aventis, Milano, Italy  

**Background**  
In the early 2000s, we identified treatment patterns of early breast cancer (EBC) in Italy (NORA study). To ascertain whether...
attitudes have changed, we conducted a similar study in 2008. Patients and methods: In this retrospective study (NEMESI), we recorded the clinical, tumour and treatment characteristics of 1.894 EBC patients in 63 Italian oncological centres in 2008, and compared the results with those of NORA, on 3.515 patients in 70 same/similar Italian institutions, in 2000-2003. Tumor stage, surgery and endocrine-responsiveness are not comparable because of different recruitment protocols, patients characteristics and adjuvant treatment strategies are perfectly comparable as a reliable picture of adjuvant strategies in Italy.

Results: There was no difference in age class distribution, menopausal status, ECOG performance status, tumor grade, ki67 and estrogen receptors content. HER2 status was simila but determined in 98% of cases in NEMESI vs 46.2% in NORA. The overall use of endocrine treatment (HT) increased from 80.3% in NORA to 83.5% in NEMESI (p = 0.005); the overall use of chemotherapy (CHT) decreased from 68.4% to 57.8% (p = 0.00001). HT alone increased from 31.0% to 42.2% (P = 0.00001) and CHT alone decreased from 18.8% to 16.2% (p = 0.017). The use of both treatments decreased from 49.2% to 41.6% (p = 0.00001). Trastuzumab was never given in NORA and in 15% of cases in NEMESI (85% of all HER2 positive cases).

The choice for HT was tamoxifen for 5 years in 32.5% of cases in NEMESI and 86.6% in NORA (p = 0.00001), and aromatase inhibitors for 5 years in 55.2% vs 13.3% of cases, respectively (P = 0.000001). The “switch” from tamoxifen 2-3 years to aromatase inhibitors 2-3 years was planned in 10.1% of cases in NEMESI and actually done in 12.9% of cases in NORA (p = 0.001). The overall use of LHRH analogues was significantly higher in NEMESI vs NORA (26.7% vs 3.0%, p = 0.00001). CMF-like regimens decreased from 37.0% in NORA to 9.1% (p = 0.000001). Anthracycline-containing regimens decreased from 52.1% in NORA to 48.8% in NEMESI, p = 0.0001). CMF-like regimens decreased from 37.0% in NORA to 9.1% (p = 0.000001). Anthracycline-containing regimens decreased from 52.1% in NORA to 48.8% in NEMESI (p = 0.00001). The use of taxanes alone regimens remained low (3.5% vs 3.7%, ns). The overall use of anthracyclines and taxanes increased from 53.0% to 87.5% and from 4.3% to 42.1%, respectively (both p = 0.00001).

Conclusions: EBC treatment has changed significantly in 6 years. Adjuvant strategies are significantly different, with a great increase of aromatase inhibitors and LHRH analogues associated with tamoxifen; anthra-taxane combinations have also significantly increased. Financial Support by Sanofi Aventis Italy

P3-01-01
Geminin Overexpression Prevents the Completion of Topoisomerase IIα Chromosome Decatenation Leading to Aneuploidy in Human Mammary Epithelial Cells.
ElShamy WM, Gardner L, Malik R, Shimizu Y, Mullins N. University of Mississippi Medical Center, Jackson, MS

Topoisomerase IIα (TopoIIα) cleaves DNA in a reversible manner, making it a valuable target for agents such as etoposide that trap the enzyme in a covalent bond with the DNA end it cleaves and prevents DNA re-ligation and triggers cell death in cancer cells. However, development of resistance to these agents limits their therapeutic use. In this study, we examined therapeutic targeting of geminin for improving the therapeutic potential of TopoIIα agents. Human mammary epithelial (HME) and breast cancer cell lines were used. Geminin, TopoIIα, Cdc7 silencing was done using specific siRNAs. Transit or stable inducible overexpression of these proteins and CKIe were also used, as well as several pharmacological inhibitors that target TopoIIα, Cdc7, or CKIe. We manipulated HME cells expressing H2B-GFP, in order to detect chromosome bridges. Immunoprecipitation and direct western blot were used to detect interactions between these proteins and their total expression, respectively, whereas interactions on chromosomal arms were detected using the TARDIS assay. TopoIIα phosphorylation by Cdc7 or CKIe was done using in vitro kinase assay. The TopoGen decatenation kit was used to measure TopoIIα decatenation activity. Finally, comet assay and metaphase chromosome spread were used to detect chromosome breakages and changes in chromosome condensation or numbers, respectively.

We found that geminin and TopoIIα interact in G2/M/early G1 cells on chromosomes, that geminin recruits TopoIIα to chromosomal decatenation sites or vice versa, and that geminin silencing in HME cells triggers the formation of chromosome bridges through suppressing TopoIIα access to chromosomal arms. CKIe kinase phosphorylates and positively regulates TopoIIα chromosome localization and function. CKIe kinase overexpression or Cdc7 kinase silencing (also phosphorylates TopoIIα in vitro), restored DNA decatenation and chromosome segregation in geminin-silenced cells before triggering cell death. In vivo, at normal concentration, geminin recruits the desumoylating enzymes SENP1 and SENP2 to desumoylate chromosomal bound TopoIIα and promote its release from chromosomes following completion of DNA decatenation. In cells overexpressing geminin, premature departure of TopoIIα from chromosomes is thought to be due to the fact that geminin recruits more of these desumoylating enzymes, or recruits them earlier, to chromosomal bound TopoIIα. This triggers premature release of TopoIIα from chromosomes, which we propose induces aneuploidy in HME cells, since chromosome breakages generated through were not sensed and/or repaired and the cell cycle was not arrested. TopoIIα recruitment and its chromosome decatenation function require normal level of geminin. Geminin silencing induces a cytokinetic checkpoint in which Cdc7 phosphorylates TopoIIα and inhibits its chromosomal recruitment and decatenation function. Geminin overexpression prematurely desumoylates TopoIIα, triggering its premature departure from chromosomes and leading to chromosomal abnormalities and the formation of aneuploid, drug resistant cancer cells. We propose that therapeutic targeting of geminin is essential for improving the therapeutic potential of TopoIIα agents.

P3-01-02
Bhaskaran SS, Kesavaram N, Nickisch KJ, VandeBerg JL, Nair HB. Texas Biomedical Research Institute, San Antonio, TX; Evestra, Inc., San Antonio, TX

Progesterone receptor (PR) is a ligand activated transcription factor which plays a crucial role in female reproduction. PR antagonists have been shown to repress estrogen dependent proliferation in the uterus and mammary gland. Even though PR antagonists are potent therapeutic candidates for breast cancer, partial agonism towards glucocorticoid receptor (GR) and androgen receptor (AR) lead to undesirable side effects. Evestra’s rational design and development approach in the synthesis of EC304 was aimed at minimizing these side effects as well as to deliver more potent and selective antiprogestins. We used select screen® (Invitrogen) to determine relative binding affinity of EC304 with AR, GR and PR. The binding affinity of EC304 showed strong antagonism towards PR with mild or negligible agonism with AR or GR. PR transactivation was inhibited by EC304 with IC50 of 0.04nM in the presence of progesterone (5nM).
EC304 showed superior cytotoxicity over known antiprogestins ZK230211, ORG33628, CDB2914, CDB4124 and RU486 in T47D cells. IC50 of EC304 was found to be 0.5nM in a 6-day cytotoxicity assay (MTT) in T47D cells stimulated with 1nM estrogen (E2). In a 21-day in vitro tumorigenicity assay, EC304 inhibited 80 and 100% of colony formation of T47D cells in the presence of E2 at 1 and 10 nM respectively. Further studies revealed that EC304 induced apoptosis and G1-phase arrest in cell cycle progression when co-incubated with E2 for 24 h. Treatment with EC304 down regulated PR target genes and up regulated cell cycle dependent kinase (CDK) inhibitor P21 in vitro. Ongoing studies address whether EC304 is specifically acting on PR-A or PR-B isoform at gene expression, protein levels and PR isoform specific knockdowns in T47D and other metastatic breast cancer cells. Considered together, these data indicate that EC304 might be a safer but efficacious novel antiprogestin for hormone sensitive postmenopausal breast cancer patients.

**P3-01-03**
The Hominoid-Specific Gene SHON Is Oncogenic in Human Mammary Carcinoma.
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Breast cancer is still the most common malignancy and the leading cause of cancer deaths in both developed and under developed countries. Molecular targeted therapy, in particular monoclonal antibody-based treatment, has emerged as a promising approach to treat this disease because of its high specificity and reduced toxicity. As a consequence, identification of novel molecular therapeutic targets is of crucial importance. In an effort to discover new targets for monoclonal antibody-based targeted therapy, we have identified a novel and secreted hominoid-specific molecule, SHON (secreted hominoid-specific oncogene), in the human mammary carcinoma cell line, MCF-7. Like other hominoid-specific genes, SHON has no known orthologs outside of the primate lineage. It is highly expressed in all cancer cell lines tested so far including breast, lung, liver, stomach, colon and prostate cancer as determined at the mRNA level by RT-PCR and at the protein level by western blotting. Forced expression of SHON in MCF-7 cells significantly increased cell proliferation and survival as demonstrated by 5-bromo-2'-deoxyuridine (BrdU) incorporation and Annexin V-propidium iodide apoptosis assays, respectively. Forced expression of SHON also promoted MCF-7 cell anchorage independent growth in soft agar and enhanced cell migration and invasion. Moreover, forced expression of SHON in MCF-7 cells increased tumour volume in a xenograft model of human breast cancer in immunodeficient mice. Furthermore, depletion of endogenous SHON expression using small interfering RNAs, or functional inhibition using an inhibitory rabbit anti-SHON polyclonal antibody, decreased MCF-7 cell proliferation and survival, and reduced MCF-7 oncogenicity and invasiveness. Therefore, SHON is a novel oncogene for mammary carcinoma cells which may be useful as a therapeutic target for the treatment of breast cancer.

**P3-01-04**
Differential Impact of Gefitinib and PLX4720 on Proliferation of MCF10A and Isogenic Lines as Measured with a Metastasis Expression Score.

Background: We previously reported that a published “Metastasis Score” (MS) could be used to evaluate the effects of a PI3K inhibitor (GDC-0941) on mutated human isogenic breast cancer cell lines. MS, based on the expression of 14 genes, has been shown to predict distant metastasis in ER(+), node (-), breast cancer. We were interested to determine the impact of gefitinib (EGFR inhibitor) and PLX-4720 (selective inhibitor of BRAF V600E) on MS to assess the applicability of this score to a broader class of targeted agents. In addition, given the cross-talk between metabolism and proliferation, we also profiled gene expression in glycolysis, fatty acid metabolism and oxidative phosphorylation.

Methods: Parental MCF10A (WT for all genes) and isogenic lines of MCF10A harboring PI3K (H1047R), p53 null or KRAS(G12V) mutations were cultured overnight in DMEM:F12 media under identical conditions. Cells were then treated with gefitinib, PLX4720, or DMSO (vehicle control) and further incubated for 24hr. Expression analysis was performed by RT-PCR.

Results: The MS of the PI3K (H1047R) and p53 null lines was higher than the parental line and lower for the KRAS (G12V) line. Treatment of parental, PI3K and KRAS cell lines with gefitinib resulted in dose-dependent decreases in MS, as reported for GDC-0941, with a higher dose required to inhibit growth of PI3K (H1047R) cells. Treatment of p53 null cells with gefitinib, however, had only a modest effect on MS. In contrast to gefitinib, MS increased with PLX-4720 treatment in all 4 lines; the greatest increase was observed in KRAS (G12V) cells. The expression of metabolic genes differed significantly depending on the oncogenic mutation harbored by the cell line. ACTA2, ACLY, RPLA, KHK, GLS2 were most highly expressed in p53 null but lowest in KRAS (G12V) cells. The enhanced proliferation of a BRAF WT cell line treated with gefitinib or PLX-4720 supports the findings that sensitivity to gefitinib requires active p53 in order to induce apoptosis through a p53-dependent pathway. Increases in MS were observed in all 4 cell lines treated with gefitinib or PLX-4720, with the largest increase observed in KRAS (G12V) cells. The expression of metabolic genes differed significantly depending on the oncogenic mutation harbored by the cell line. ACTA2, ACLY, RPLA, KHK, GLS2 were most highly expressed in p53 null but lowest in KRAS (G12V) cells. The enhanced proliferation of a BRAF WT cell line treated with gefitinib supports the impact of this EGFR inhibitor on cell proliferation. The modest effect on MS in p53 null cells supports the findings that sensitivity to gefitinib requires active p53 in order to induce apoptosis through a p53-dependent pathway. Increases in MS were observed in all 4 cell lines treated with PLX-4720, with the largest increase observed in KRAS (G12V). The enhanced proliferation of a BRAF WT cell line with a KRAS mutation is consistent with the Ras-dependent nature of this pathway. The metabolic genes showed diverse expression patterns that differed with different oncogenic mutations and likely reflect the multiple mechanisms controlling metabolism in cancer. An improved understanding of the expression of metabolic genes relative to proliferation in cell lines with various oncogenic mutations may provide additional insights into the dysregulation of these cellular processes and the possible role of anti-metabolite intervention.
Wnt Signaling: A New Target for Treatment and Prevention of Endocrine Resistant Breast Cancer?
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Background: Wnt signaling is altered in nearly half of all breast cancers. Both up regulation of Wnt pathway activators and down regulation of pathway inhibitors have been identified in breast cancer. Previously we have reported that activity of β-catenin is altered in acquired tamoxifen-resistant (TamR) breast cancer cells compared to endocrine–sensitive parental MCF7 cells, with an increase in β-catenin-mediated gene transcription. Data is lacking on the role of Wnt signaling in acquired endocrine resistance in breast cancer. Endocrine relapse predicts a poor clinical outcome and remains an important target for future drug development. In this study we investigated the role of Wnt signaling in acquired tamoxifen resistance and its contribution to the associated aggressive phenotype in vitro.

Materials/ Methods: We performed exploratory Affymetrix HGU-133A gene microarray analysis of key Wnt pathway components in log-phase TamR vs. MCF7 cell lines, as well as comparing MCF7 cells +/- 10 day tamoxifen exposure. This was paralleled by western blot (WB) analysis. Effects of Wnt signaling inhibition on growth and cell migration were explored using two commercially available inhibitors: IWP2 (inhibits Wnt production, pLRP6) and PNU74654 (interferes with β-catenin-Tcf/Lef binding). MTT cell proliferation assays (6 days) and cell migration assays (24hrs) were carried out. Results: Microarray analysis of key Wnt pathway components revealed up regulation of wnts 3, 4 and 6; up regulation of Wnt pathway activators LRP5 and LRP6; down regulation of pathway inhibitors (DKK1, DKK4); and down regulation of Axin1 (destruction complex component). Changes are >1.5-fold. WB analysis confirmed up regulation of total β-catenin and LRP6 in TamR versus MCF7 cells as well as activity of LRP6. In accordance with this, inhibition of Wnt signaling using IWP2 10uM or PNU74654 10uM, whilst having no effect on MCF7 cell proliferation, significantly suppressed the growth of TamR cells (p<0.001). Inhibition of Wnt signaling in TamR cells also suppressed cell migration (p<0.001). Gene microarray data for MCF7 cells treated with tamoxifen for 10 days showed up regulation of LRP6 and wnt4 and down regulation of LRP5, wnt6, Axin1 and DKK4 compared to control.

Discussion: These observations suggest that deregulated Wnt signaling may play a role in acquired tamoxifen resistance in breast cancer where it may act to promote growth and the development of a more aggressive phenotype. Its interaction with other pathways is important in the prevention of resistance with co-treatment strategies. Wnt1 can rescue MCF7 cells from growth suppression caused by 4-hydroxytamoxifen. Given the early changes in the Wnt pathway in MCF7 cells treated with tamoxifen, further exploration in the context of earlier Wnt targeting alongside anthornabone treatment is required. Monitoring of Wnt signaling within clinical samples from patients who have relapsed after hormone treatment is urgently warranted given that the Wnt inhibitors showed promise in controlling this state in vitro.

NF-κB Inhibition Promotes Radiosensitivity of Breast Cancer Cells in Three-Dimensional Culture through Abating β1-Integrin Expression.
Ahmed KM, Zhang H, Park CC. Ernest Orlando Lawrence Berkeley National Laboratory, Berkeley, CA

Therapy-associated tumor resistance, giving rise to recurrence and mortality, is a critical issue in cancer therapy. The molecular mechanisms causing tumor resilience to the therapeutic radiation remain elusive. Nuclear factor-KB (NF-KB), a stress-sensitive heterodimeric transcription factor in the regulation of the stress-responsive genes, has been shown to initiate prosurvival signaling pathways. The cooperative function of NF-KB with other key stress elements in radiosensitivity remains to be elucidated. We have previously reported that induction of α5β1-integrin is associated with the enhanced cell survival of breast cancer cells after exposure to high dose IR (ionizing radiation). Because a typical NF-KB binding site was located in human β1-integrin promoter region, β1-integrin-mediated resistance to radiation may be regulated by NF-KB. The aim of the present study was to reveal a connection between NF-KB and β1-integrin pathways in radioprotection of malignant T4-2 mammary epithelial cells in 3D IrECM (three-dimensional laminin-rich extracellular matrix). We show that the elevated NF-KB activity was correlated with enhanced clonogenic survival, and increased NF-KB heterodimer p50/p65 levels were associated with an increase in total and phosphorylated (Thr 788/789) β1-integrins. Inhibition of NF-KB activation significantly reduced clonogenic survival with the inhibition of β1-integrin. These results indicate that NF-KB-mediated induction of β1-integrin is associated with an increased radiation resistance. Treatment of T4-2 colonies, formed at day 4, with NF-KB activation inhibitor in 3D IrECM before exposure to IR (4-Gy X-ray) resulted in a reduction of the size of colonies. The surviving colonies were associated with a decrease in proliferation and increase in apoptosis, indicating a decrease of resistance to IR. Together, these results provide the first evidence that NF-KB-mediated β1-integrin expression is responsible for tumor radiosensitivity. The NF-KB/β1-integrin pathway may serve as an efficient drug target to re-sensitize radiosensitive tumor cells.

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BACKGROUND: ciclooxygenase-2 (COX-2) has been suggested as a necessary component of the cellular and molecular mechanisms behind breast cancer cell motility and invasion. The potential therapeutic benefit of COX-2 inhibitors in a range of cancers is being seen as a great promise however, there have been recent concerns about potential cardiotoxicity. Thus, there is an urgency to develop new inhibitors exhibiting a better risk/benefit ratio. Acyl-CoA synthetase 4 (ACSL4), belongs to a five-member family of enzymes that esterifies mainly arachidonic acid into acyl-CoA. We have provided first-time evidence demonstrating that, ACSL4 is the key enzyme that regulates the induction of COX-2, the production of prostaglandin E2 (PGE2) and the proliferation and metastatic potential of breast cancer cells. Therefore, the aim of the study was...
the development of an in vivo model of human breast tumor xenografts for the study of the regulation of COX-2 expression and action.

RESULTS: stable transfection of MCF-7 cells with ACSL4 cDNA under the control of tetracycline (MCF-7 Tet-off-ACSL4) resulted in a significant increase in the expression of COX-2. The increment in COX-2 expression registered in MCF-7 Tet-off-ACSL4 cells is accompanied with an increase in the production of PGE₂, and the proliferation and metastatic potential of cancer cells. Next, we tested whether the injection of MCF-7 Tet-off/ACSL4 cells into nude mice resulted in tumor development.

The results of those experiments demonstrate that MCF-7 Tet-off/ACSL4 cells develop into murine mammary tumors whereas cells transfected with the MCF-7 Tet-off empty vector did not. Interestingly, treatment of nude mice with tetracycline resulted in tumor growth inhibition. The results show that the sole transfection of ACSL4 results in a phenotype change that endows the cells with the capacity to develop into tumors when injected into nude mice. Tumor volume (TV) at day 70 reached 468.4±189.3mm³. Treatment with tetracycline reduced TV to 189.3±65.9mm³ (p<0.05). The growth rate between days 45-70 was 12.30 mm³/day. Tumors were classified as Basal-like subtypes.

We have also observed an inhibition of proliferation and migration of MDA-MB-231 cell cultures when exposed to a combination of ACSL4 and COX-2 inhibitors. Interestingly, the compounds assayed markedly reduced cell proliferation and migration at concentrations that are less effective when used alone. These results point to an effect that has the advantage of exposing the cells to lower drug concentrations.

CONCLUSION: Based on our results, we hypothesize that ACSL4 and COX-2 could constitute potential therapeutic targets for the control of tumor growth. Our animal model of mammary tumors constitutes a proper platform for the study of those therapies.

P3-01-08

In Vitro and In Vivo Antitumor Activity of the Aurora and Angiogenic Kinase Inhibitor ENMD-2076 in Triple-Negative Breast Cancer Models.

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Background: Triple-negative breast cancer (TNBC) is an aggressive biologic subtype which lacks effective targeted anti-cancer agents and is characterized by a high mitotic index and dependence on angiogenesis. ENMD-2076 is a novel orally bioavailable Aurora and angiogenic kinase inhibitor currently in clinical development with favorable pharmacokinetics and a manageable toxicity profile. The purpose of this study was to use TNBC cell line-based in vitro and in vivo models to demonstrate the antitumor activity of ENMD-2076 towards this breast cancer subtype compared to the luminal and HER2-amplified subtypes. Using significance of analysis of microarrays (SAM) analysis and GSEA, we identified Ran, a member of the mitotic spindle regulation pathway as upregulated in sensitive TNBC cell lines (p = 0.017). Interestingly, AURKA, the main target of ENMD-2076, is a core gene in this pathway.

Conclusions: ENMD-2076 exhibited robust anticancer activity towards preclinical models of TNBC, supporting future clinical investigations of this agent in TNBC with an emphasis on the continued development of biomarkers predictive of response in this breast cancer subset.

P3-01-09

Oncogenic Activation of HSF1 Enables the Malignant Progression of Breast Carcinoma.

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HSF1 is best known for its role as the master transcriptional regulator of the evolutionarily conserved heat-shock response. In mice, Hsf1 knock-outs dramatically reduce susceptibility to malignant transformation and tumor formation, and markedly increased survival in cancers driven by both oncogenic and tumor suppressor mutations. Likewise, RNAi-mediated knockdown markedly reduces the growth and survival of human cell lines established from cancers driven by a diversity of genetic lesions. The transcriptional network that HSF1 coordinates during heat-shock is known, but is unknown in malignancy. We compare HSF1 function in isogenic breast cancer cells of high and low malignant potential. HSF1 orchestrates a far-reaching transcriptional program that is dependent on transformation and the degree of malignancy. The oncogenic HSF1 program differs markedly from the classical heat-shock response. It is enriched for genes involved in a myriad of cellular processes known to be important in malignancy, including transcription, translation, glucose metabolism and cellular adhesion. It includes only a particular subset of heat-shock protein genes, and many of these are regulated in a manner that differs from their regulation by heat-shock. We also find that HSF1 is overexpressed and activated in a large subset of all conventionally defined classes of breast cancer. Moreover, tumors with high expression of the oncogenic HSF1 transcriptional network
are strongly associated with poor outcome as monitored by metastasis and death. Our findings suggest that the oncopgenic HSF1 activation program is rooted in fundamental aspects of tumor biology and will prove a powerful new tool in the clinical management of patients with breast cancer and, likely, other malignancies as well.

**P3-01-10**

**DMBA-Breast Cancer in Diet Induced Obesity (DIO) and Lean Mice Is Related to Leptin Signaling.**

Gillespie C, Penichet MG, Colbert LS, McGlothlen T, Guo S, Zhou W, Gonzalez-Perez RR. Morehouse School of Medicine, Atlanta, GA; Shenyang Medical College, Liaoning Pro, P.R., China

**Introduction:** Leptin, the major adipokine released by adipose tissue, is strongly related to obesity-related cancers. We have shown previously that inhibition of leptin signaling with pegylated leptin peptide receptor antagonist 2 (PEG-LPrA2) negatively impacted human xenograft and syngeneic mouse breast cancer growth as well as VEGF/VEGFR2 levels [1,2].

**Objective:** We hypothesize that leptin signaling is essential for 7,12-dimethylbenz[A]anthracene (DMBA)-mammary tumor (MT) particularly in obese mice.

**Methods:** The study investigated the potential of PEG-LPrA2 to prevent MT in lean and diet-induced-obesity (DIO)-C57BL/6J female mice treated with 1 mg/dose/weekly for 6 weeks of DMBa. Obesity was induced by feeding DIO-mice (95% obese mice after 5 weeks) with high fat diet (PDI-1; 45% Kcal from fat). Lean mice were fed a normal diet (PDI-1; 5% Kcal from fat). PEG-LPrA2 was administered intravenously. Lean and DIO-mice received either one or two PEG-LPrA2 dose/week (50 μl/0.1 mM) two weeks prior to DMBa to test its preventative potential through the end point (32 weeks). Control mice received saline injections. **Results:** Obesity was positively correlated to the development of DMBA-MT in mice. MTs were found in 17% of lean control and 69% of DIO-control mice. Notably, PEG-LPrA2 prevented the onset of DMBA-MT in lean (one and two doses: 0% tumor-bearing mice) and DIO-mice (one-dose, 29% and two-dose 0% MT). PEG-LPrA2 treatment did not alter body weight nor food intake in lean or DIO-mice. VEGF levels were significant higher (32 fold) in control DIO-mice. PEG-LPrA2 inhibition of leptin signaling decreased tumor levels of Notch ligands (Jagged-1 & DLL-4), receptors (Notch 1-4) and target genes (Survivin and Hey2) and reduced OB-R, IL-1Rt, VEGF/VEGFR2, bcl-2, HIF-1a and NFkB in 95% of lean and 69% of DIO-control mice. Notably, PEG-LPrA2 prevented the onset of DMBA-MT in lean (one and two doses: 0% tumor-bearing mice) and DIO-mice (one-dose, 29% and two-dose 0% MT). PEG-LPrA2 treatment did not alter body weight nor food intake in lean or DIO-mice. VEGF levels were significant higher (32 fold) in control DIO-mice. PEG-LPrA2 inhibition of leptin signaling decreased tumor levels of Notch ligands (Jagged-1 & DLL-4), receptors (Notch 1-4) and target genes (Survivin and Hey2) and reduced OB-R, IL-1Rt, VEGF/VEGFR2, bcl-2, HIF-1a and NFkB in 95% of lean and 69% of DIO-control mice.

**Conclusions:** Present data strongly suggest that leptin signaling is essential for DMBA-induced MT in the context of obesity. Overall, the effective chemoprevention of DMBA-MT by PEG-LPrA2 treatment in DIO and lean mice reinforces the potential use of leptin signaling inhibition for breast cancer prevention. These observations are most significant for obese populations showing higher levels of leptin and incidence of breast cancer. [This work was supported in part by NIH/NCI SC1CA138658-02; NIH/ARRA/3SC1CA138658-02S1 and the Georgia Cancer Coalition Distinguished Cancer Scholar Award (to RRGP); CREDO (MSCR) 2R25RR017694-06A1 to L.S.C; the Morehouse School of Medicine (MSM) MBRS RISE Program (NIH/NIGMS S06 GM08248) to TZM; and facilities and support services at Morehouse School of Medicine (NIH RR03034 and JCO6 RR18386).]

References:


**P3-01-11**

**Increased Gene Copy Number of c-KIT and VEGFR2 at 4q12 in Primary Breast Cancer Is Related to an Aggressive Phenotype and Impaired Prognosis.**


**Introduction:** Triple-negative breast cancer (TNBC) accounts for approximately 15% of all female breast cancer and is associated with aggressive clinical behaviour. No targeted treatments are available for TNBC. Drugs inhibiting tyrosine kinases, such as vascular endothelial growth factor receptor 2 (VEGFR2) and c-KIT, have however shown some promising results for patients with TNBC. The aim of the present study was to investigate whether gains and/or amplifications of VEGFR2 and c-KIT occur in TNBC. These genes may constitute novel candidate biomarkers for selecting TNBC patients for treatment with tyrosine kinase inhibitors.

**Material & Methods:** Fluorescence in situ hybridization (FISH) was used to quantify gene copy numbers of VEGFR2 and c-KIT in 83 primary human breast cancers, of which 31 were classified as TNBC. Gains were defined as > 4 copy numbers in more than 40% of the cancer cells, while amplification was defined as gene copy/CEP ratio > 2 in more than 10% of the cancer cells. A tumour was considered FISH positive for c-KIT and/or VEGFR2 if it displayed copy number gain and/or amplification. Immunohistochemical (IHC) staining was performed for assessment of c-KIT protein expression.

**Results:** Ten (32%) of the TNBCs were VEGFR2 FISH positive and nine (29%) were c-KIT FISH positive, whereas non-TNBCs were FISH positive for VEGFR2 and c-KIT in nine (18%) cases for both genes. No significant difference in frequency between TNBCs and non-TNBCs was found. There was a correlation between FISH positivity for VEGFR2 and c-KIT (c² test, P<0.001), and VEGFR2 and c-KIT FISH positivity correlated to ER/PgR negativity and high Nottingham histological grade (NHG). A significantly worse breast cancer specific survival (BCSS) was seen for FISH positive cases in the whole cohort as well as among untreated patients and non-TNBCs, but not among TNBC cases.

**Discussion:** The high correlation between VEGFR2 and c-KIT FISH positivity suggests that the genes are co-amplified. Increased copy number of both genes was related to aggressive disease and a worse prognosis, and thus has the potential of functioning as a novel predictive biomarker for selected targeted therapy particularly in the difficult-to-treat TNBC patient category.

**P3-01-12**

**Prognostic Impact of RANK, RANKL and OPG Gene Expression in ER Positive Primary Breast Cancer.**

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**Background:** The cell surface receptor RANK (receptor activator of NFκB), its ligand (RANKL) and the decoy receptor of RANKL osteoprotegerin (OPG) play an important functional role in bone physiology and in the survival of cancer cells. RANKL expression is positively correlated with cytotoxicity of breast cancer cells. We investigated whether expression of RANK, RANKL and OPG were associated with clinical breast cancer patient characteristics.

**Material & Methods:** We investigated human breast cancer samples obtained from four different patient populations: breast cancer patients with estrogen receptor positive (ER+) disease, patients with breast cancer with triple negative phenotype (TNBC) and patients with breast cancer who had received neoadjuvant chemotherapy (NAC) and patients who had received metastatic breast cancer (MBC). 116 ER+ and 58 TNBC breast cancer samples were collected. For the NAC and the MBC patients, the expression levels of RANK, RANKL, and OPG were measured.

**Results:** In the ER+ breast cancer samples, the expression of RANK and RANKL was associated with metastasis. More specifically, multivariate analysis showed that the expression of RANK and RANKL was significantly associated with the number of metastatic lymph nodes (P=0.001 and 0.002). The expression of RANK and RANKL was also associated with recurrence (P=0.003 and 0.001) and metastasis (P=0.001 and 0.001) in the ER+ breast cancer samples. Interestingly, the expression of RANK and RANKL was also associated with the HER2 status (P=0.002 and 0.001) and the number of metastatic lymph nodes (P=0.001 and 0.002) in the TNBC breast cancer samples. Multivariate analysis showed that the expression of RANK and RANKL was significantly associated with the number of metastatic lymph nodes (P=0.001 and 0.001) in the TNBC breast cancer samples. The expression of RANK and RANKL was also associated with recurrence (P=0.001 and 0.001) and metastasis (P=0.001 and 0.001) in the TNBC breast cancer samples. In addition, the expression of RANK and RANKL was also associated with the HER2 status (P=0.002 and 0.001) and the number of metastatic lymph nodes (P=0.001 and 0.001) in the ER+ breast cancer samples.

**Discussion:** Our findings suggest that the expression of RANK and RANKL is associated with clinical breast cancer patient characteristics. These results support the hypothesis that RANK and RANKL expression may be used as prognostic markers in breast cancer patients.
bone metastasis by regulating osteoclasts. Just recently it was shown that tumor-infiltrating lymphocytes can stimulate breast cancer metastases through RANK-RANKL signalling.

Material and methods:
We analyzed gene expression of RANK, RANKL and OPG in a combined Affymetrix dataset of 307 ER positive breast cancers from our institutions which were either untreated or treated with chemotherapy. Kaplan Meier analysis of disease free survival and Cox regression analysis was applied to examine the prognostic value of the different markers.

Results:
We observed no significant difference in survival when samples were analyzed according to either RANK or RANKL mRNA expression. In contrast when samples were stratified in quartiles of OPG expression a positive linear relationship of survival with the expression of OPG was observed. Moreover since OPG demonstrated a bimodal type of expression a cutoff value can be derived from the expression data. Using this cutoff value a hazard ration of 2.14 (95% CI 1.27-3.61; P=0.004) for low OPG expression was detected. OPG expression correlated with lower proportion of grade 3 tumors (15.7% vs 27%; P=0.022) and a higher proportion of PgR positive samples (86.2% vs 71.4%; P=0.002). No significant differences were observed for lymph node status, age, tumor size and HER2 status. In multivariate analysis only lymph node status remained significant while OPG, Ki67, age, grade, and PgR only displayed a trend towards significance.

Conclusion:
Expression of osteoprotegerin seems to correlate with good prognosis in ER positive breast cancer. These data are in line with in vitro studies demonstration that OPG inhibits RANKL induced migration of tumor cells.

P3-01-14
RANK and RANK Ligand (RANKL) Expression in Invasive Breast Carcinoma and Human Breast Cancer Cell Lines.

Purpose:
RANK and its ligand (RANKL), key factors for bone remodeling and metastasis, are crucial for the development of mouse mammary gland during pregnancy. RANKL functions as a major paracrine effector of the mitogenic action of progesterone in mouse mammary epithelium and has a role in ovarian hormone-dependent expansion and regenerative potential of mammary stem cells (MaSC). RANKL inhibition has been shown to reduce mammary tumor formation and pulmonary metastases in mouse models. Many published expression analyses of RANK and RANKL have been performed using immunohistochemistry (IHC) without documented validation of antibody specificity. This study assessed the expression of human RANK and RANKL in human invasive breast carcinoma (IBC) and human breast cancer cell lines using specific, monoclonal antibodies validated and optimized for IHC or flow cytometry.

Methods: RANK and RANKL expression was analyzed in a panel of human breast cancer cell lines representing luminal or basal breast subtypes using qPCR, flow cytometry and surface receptor quantitation. Antibodies against human RANK (N-1H8, N-2B10; Amgen) and human RANKL (M366, AMG161; Amgen) were used for flow cytometry, surface receptor quantitation or IHC staining. For human IBC, the intensity of IHC staining was scored on a semiquantitative scale (0=absent, 1=weak, 2=moderate, 3=intense). Incidence was scored as a positive IHC signal (any intensity). In vitro responses of cell lines to RANKL were also tested.

Results: The specificity of the antibodies was substantiated by concordant signals observed using multiple independent analyses, including IHC, flow cytometry and Western blots of positive and negative control cells and xenograft samples. Analysis of primary human IBC using IHC demonstrated that 25/114 (22%) IBC samples expressed RANK and 18/97 IBC (19%) expressed RANKL protein within the tumor epithelium. RANK protein was observed in mononucelar cells infiltrating the tumor in 87/114 (76%) and within normal mammary epithelium adjacent to tumors in 35/79 (44%) of samples. RANKL was observed in infiltrating mononucelar cells within

www.aacrjournals.org 339s  Cancer Res; 71(24 Suppl.) December 15, 2011
the tumor in 60/115 (52%) and within normal mammary epithelium adjacent to tumors in 15/68 (22%) of samples. Both mRNA and RANK surface protein were detected in multiple breast cancer cell lines, including basal and luminal subtypes. Functional RANK expression on cell lines was confirmed by the observation of RANKL-dependent increases in mRNAs (e.g. MMP-9, IL-6 or IL-8) or proteins in conditioned media (e.g. IL-6, IL-8), despite the relatively low surface expression of RANK observed (range = 1240-9120 sites/cell).

**Conclusion:** RANK and RANKL expression was observed in the epithelial carcinoma element in human IBC using IHC. RANK and RANKL expression was also observed in normal mammary epithelium and monocytic cells adjacent to breast tumors. Functional RANK expression was observed in human breast cancer cell lines, including both basal and luminal subtypes.

**P3-01-15**

The Role of Src Homology Phosphotyrosyl Phosphatase-2 in Basal-Type/Triple-Negative Breast Cancer – Implications for Targeted Therapy.

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Background: Of all breast cancer subtypes, the basal-type/triple-negative breast cancer (BTBC) has the worst clinical outcome. The major reasons are the highly invasive nature of the disease and the limited treatment options available to patients. As opposed to the HER2-positive and the hormone-positive breast cancers, targeted therapy against BTBC is unavailable. These premises underscore the need for discovering and characterizing drug targets in BTBC.

In this study, we have focused on the Src homology phosphotyrosyl phosphatase 2 (SHP2) which seems to play pivotal roles in BTBC. The major reason for focusing on SHP2 is that it is an essential downstream effector of mitogenic and cell survival signaling downstream of receptor tyrosine kinases such as EGFR (HER1) and IGF-1R, and the cytoplasmic tyrosine kinase Src, which are known to be elevated in BTBC.

Materials and Methods: The state of SHP2 and EGFR expression in BTBC tumors was determined by immunohistochemistry. The functional significance of SHP2 in BTBC cells was investigated by ablating its expression with specific siRNA and then assessing impact on mitogenic and cell survival signaling using immunoblotting with phospho-specific antibodies, effect on transformation using 3D cultures such as growth in soft agar and matrigel, consequence on cell motility and invasiveness using flow-cytometry-tagged matrigel. Furthermore, the impact of SHP2 inhibition on tumorigenesis was tested by intramammary transplantation in vivo and monitoring and analyzing tumor growth and metastasis.

Results: We have shown that the SHP2 protein is elevated in BTBC tumors. More importantly, the elevated expression of SHP2 is highly correlated with overexpression of the EGFR (HER1), suggesting their potential synergistic role to promote BTBC. Inhibition of SHP2 in BTBC cells reversed transformation and suppressed proliferation, indicating their dependency on the function of SHP2. Moreover, inhibition of SHP2 abolished EGF-induced mitogenic and cell survival signaling, which is in agreement with its role in cell proliferation and transformation. More dramatic was that the invasive phenotype of BTBC cells in 3D matrigel was completely blocked by inhibition of SHP2, suggesting that the invasive property of these cells is dependent on SHP2. Even more dramatic was that the tumorigenic and metastatic potential of BTBC cells was abolished by SHP2 inhibition.

Discussion: The current study demonstrates that SHP2 plays a pivotal role in promoting BTBC. Given that SHP2 is a tyrosine phosphatase with positive signaling role, its promotion of BTBC must occurs through promotion of tyrosine kinase signaling. Together, our results provide the first glimpse on the potential of SHP2 as a drug target in BTBC.

**P3-01-16**

Expression of Basal Markers in Correlation with PTEN and BRCA 1 in Triple Negative Breast Carcinomas (TN) Treated with Chemotherapy Versus PARP Inhibitors.

Chivukula M, Carter G, Puthalla S, Magee Women’s Hospital of Pittsburgh, Pittsburgh, PA; Magee Women’s Hospital of UPMC, Pittsburgh, PA

The link between the BRCA1 tumour-suppressor gene and hereditary breast and ovarian cancer is established. The morphologic and immunohistochemical features of basal like phenotype carcinomas have shown to be correlated with the BRCA 1 expression. PTEN (phosphatase and Tensin Homolog) is a novel tumour suppressor gene located on chromosome 10. PTEN mutations are believed to exert their effects through the putative PI3K-AKT-mTOR signaling pathway. Lack of PTEN expression, suggests that PARP inhibitors may be therapeutically useful for a subset of invasive breast cancers. The data on PTEN status in breast carcinomas is emerging. The aim of our study is to assess the immunohistochemical expression of basal markers, PTEN and BRCA 1 in a subset of triple negative breast carcinomas with known BRCA status. Results: Results are shown in Table 1.

**TABLE 1**

<table>
<thead>
<tr>
<th>Basal Marker</th>
<th>TN Chemo+ (n=17)</th>
<th><strong>TN PARP+ (n=6)</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>CKSC (+)</td>
<td>12/17</td>
<td>6/6</td>
</tr>
<tr>
<td>CK 5 (+)</td>
<td>5/17</td>
<td>6/6</td>
</tr>
<tr>
<td>EGFR (+)</td>
<td>16/17</td>
<td>6/6</td>
</tr>
<tr>
<td>BRCA 1 (+)</td>
<td>15/17</td>
<td>6/6</td>
</tr>
<tr>
<td>PTEN (+)</td>
<td>15/17</td>
<td>6/6</td>
</tr>
<tr>
<td>PTEN (-)</td>
<td>0/6</td>
<td></td>
</tr>
<tr>
<td><strong>TN, Chemo+ (n=17)</strong></td>
<td></td>
<td><strong>TN, PARP+ (n=6)</strong></td>
</tr>
</tbody>
</table>

TN: triple negative; Chemo: patients who received chemotherapy; (**): Study ongoing with new patients; (+): Positive; (-): Negative

Conclusions: 1. Initial results from our study indicate high expression of PTEN is seen in TN breast carcinomas. 2. There appears to be decreased BRCA 1 protein expression in TN patients who tested positive for BRCA mutations. 3. Given the loss of PTEN in a subset of TN Chemo group, PARP inhibitors may be suggested to be therapeutically useful and needs to be evaluated further. 4. The lack of CK 5 basal keratin in some patients needs to be addressed further. The study is on going with addition of new cases. The updated data and preliminary data with patient responses on PARP inhibitor will be presented.

**P3-02-01**

A Novel Bispecific, Hexavalent, Antibody (HexAb) Inhibits Anchorage-Independent Growth and Reduces Invasiveness of Triple-Negative Breast Cancer Cell Lines In Vitro.


Background: Combination therapy using two different monoclonal antibodies to achieve improved efficacy without increased toxicity
is being pursued in various preclinical and clinical studies but, in principle, could be accomplished with a single bspecific antibody (bsAb). We note the elevated expression of the type-I insulin-like growth factor receptor (IGF-1R) and the trophoblast cell-surface marker (Trop-2) in diverse epithelial tumors, including breast cancers, and the increasing evidence of their correlation with poor survival. Therefore, we chose to explore the potential of 1R-(E1)-(E1), the bspecific HexAb constructed from hR1 (humanized anti-IGF-1R) and hRS7 (humanized anti-Trop-2) for treating breast cancers. Methods: 1R-(E1)-(E1) was generated by the Dock-and-Lock (DNL) method to comprise a full IgG of hR1 linked to two stabilized dimers of hRS7 Fab, and evaluated in three HER2-negative breast cancer cell lines: the non-evasive MCF-7, the moderately invasive MDA-MB-468, and the highly invasive MDA-MB-231. The in vitro characterizations of 1R-(E1)-(E1) included flow cytometry to determine cell binding, soft agar assay to evaluate the effect on anchorage-independent growth, and BD matrigel chambers to assess invasion properties. Statistical differences (P values) between two populations were determined by Student's t-test. Results: All three cell lines were found to express both IGF-1R and Trop-2, with MCF-7 and MDA-MB-468 notably higher in IGF-1R and Trop-2, respectively. As expected, 1R-(E1)-(E1) bound to all three cell lines. At 50 μg/ml, 1R-(E1)-(E1) reduced the invasion of MDA-MB-468 to less than 10% of the untreated control, whereas under the same conditions, MDA-MB-231 appeared to be resistant, while the parental antibodies showed no effects. The ability of 1R-(E1)-(E1) to inhibit anchorage-independent growth was demonstrated at 200 nM in MDA-MB-231, with a statistically significant difference (P=0.041) when compared with samples treated with parental antibodies at the same concentrations. Cells treated with 1R-(E1)-(E1) produced few and much smaller colonies, the largest size of which was less than 1/10 of the untreated cells. The parental hR1 alone, but not hRS7, had some effect on inhibiting the growth of MDA-MB-231 in soft agar, presumably resulting from the down-regulation of IGF-1R. Conclusions: These promising results warrant further evaluation of 1R-(E1)-(E1) in other breast cancer cell lines, as well as the exploration of new bspecific HexAbs that comprise different antibodies capable of targeting other solid cancers.

P3-02-02
Novel Ranpirnase-Based ImmunoRNases Display Potent Cytotoxicity in Diverse Human Breast Cancer Cell Lines.

Ranpirnase (Rap) is an amphibian ribonuclease originally isolated from the oocytes of Rana pipiens. Rap shows anti-tumor activity in diverse cancers and its potency can be enhanced by chemically linking or recombinantly fusing Rap to a tumor-targeting antibody, as demonstrated for CD22- or CD74-expressing hematological malignancies, as well as a variety of Trop-2-expressing cell lines derived from breast, cervical, lung, pancreatic, and prostate cancers. The Dock-and-Lock (DNL) platform enables the design and generation of targetable therapeutics that are multivalent, multispecific, and multifunctional. Here, we report the successful application of the DNL method to generate a novel class of Rap-based immunomRNases, each of which features a pair of dimeric Rap molecules covalently tethered to a select monoclonal antibody at the carboxyl termini of the heavy chains. Two such constructs, designated E1-Rap and 22-Rap, were developed with hRS7 (humanized anti-Trop-2) and epratuzumab (humanized anti-CD22), respectively, purified to near homogeneity, and evaluated in a panel of human breast cancer lines, including the basal-like, triple-negative subtype (MDA-MB-468, MDA-MB-231, BT20, HCC1806, and HCC1395), the luminal B, HER2-negative subtype (MCF-7), and the HER2-positive subtype (SKBR3), all except HCC1395 expressing high to moderate levels of Trop-2, and none expressing CD22. As demonstrated by flow cytometry, E1-Rap and hRS7 bound equivalently to MDA-MB-468, indicating the affinity of E1-Rap for Trop-2 is not compromised. Surprisingly, 22-Rap, but not epratuzumab, also bound substantially to MDA-MB-468, albeit to a lesser extent than E1-Rap. We thus postulate that the four highly basic Rap molecules in the DNL conjugate may confer a spatial configuration to largely enhance their interaction with negatively-charged cell surface proteins, such as heparan sulfate proteoglycans. Whereas the individual DNL component (IgG or Rap) alone or in combination showed negligible in vitro cytotoxicity in all seven breast cancer cell lines examined, E1-Rap exhibited EC50 values of 1 nM or less in MDA-MB-468 (0.03 nM), MCF-7 (0.1 nM), BT20 (0.18 nM), HCC1806 (0.19 nM), and SKBR3 (1.29 nM). In comparison, the potency of 22-Rap was at least 10-fold lower than E1-Rap in MDA-MB-468, BT20 and HCC1806, with an EC50 of ~2 nM. Neither E1-Rap nor 22-Rap was very effective in inhibiting the proliferation of the more aggressive MDA-MB-231, with an EC50 above 50 nM. In the Trop-2-negative HCC1395, the dose-response curves obtained for E1-Rap and 22-Rap were nearly identical (EC50~100 nM), as they should be if the cytotoxicity was mediated mainly through the Rap component. The results of Immunofluorescence microscopy showed E1-Rap was effectively internalized and localized in the cytosol. Thus, these ImmunoRNases are potentially new cancer therapeutics for breast and other solid tumors.

P3-03-01
Toyama T, Kondo N, Endo Y, Sugiuira H, Yoshimoto N, Iwasa M, Takahashi S, Iwase H, Fujii Y, Yamashita H. Nagoya City University Graduate School of Medical Sciences, Nagoya, Aichi, Japan; Kumamoto University Graduate School of Medical Sciences, Kumamoto, Japan

Background: microRNAs (miRNAs) have emerged as a new class of non-coding genes involved in regulating cell proliferation, differentiation, and viability. Recent studies have identified miR-210 among a set of hypoxia-regulated miRNAs and demonstrated a direct regulatory role of hypoxia-inducible factor-1 alpha (HIF-1α) in its transcription. High expression of miR-210 has been reported to be a poor prognostic factor in several types of cancers including breast. Materials and Methods: TaqMan MicroRNA assays for miR-210 expression were performed in 219 breast cancers (58 triple-negative TNBCs), and 161 ER-positive and HER2-negative. Correlations between miR-210 expression and clinicopathological factors were analyzed. The effects of several variables on survival were tested by Cox proportional hazards regression analysis. Results: miR-210 expression in TNBCs was significantly higher than in ER-positive and HER2-negative breast cancers (p<0.001). Patients whose TNBCs had low miR-210 expression experienced significantly better disease-free and overall survival compared with high miR-210 expressors (p=0.02 and p=0.05, respectively). Notably, among 40 node-negative TNBCs, 5-year disease-free survival was approximately 60% in patients whose tumors had high or intermediate miR-210 expression (n=26), while no patients with low miR-210 expression (n=14) suffered recurrent disease. Cox univariate and
multivariate analyses demonstrated that low expression of miR-210 was an independent good prognostic factor in TNBCs. miR-210 expression in breast cancer: Cox proportional hazards regression analysis in breast cancer patients: Cox proportional hazards regression analysis

<table>
<thead>
<tr>
<th>miR-210</th>
<th>p value</th>
<th>p value</th>
<th>risk ratio of recurrence (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low expression</td>
<td>0.0</td>
<td>0.0</td>
<td>1.0 (reference)</td>
</tr>
<tr>
<td>Intermediate or High expression</td>
<td>0.036</td>
<td>0.049</td>
<td>4.39 (1.00 to 19.28)</td>
</tr>
<tr>
<td>Nodal status</td>
<td>0.096</td>
<td>0.078</td>
<td>1.0 (reference)</td>
</tr>
<tr>
<td>Negative</td>
<td>0.0</td>
<td>0.0</td>
<td>1.0 (reference)</td>
</tr>
<tr>
<td>Positive</td>
<td>0.1</td>
<td>0.1</td>
<td>1.0 (reference)</td>
</tr>
</tbody>
</table>

Discussion: Although prognosis of patients with TNBCs is poor, those whose tumors expressed low levels of miR-210 had a more favorable prognosis. Thus, the degree of miR-210 expression might be a clinically useful prognostic factor for decision-making regarding treatment in the adjuvant setting, especially in node-negative TNBC patients.

P3-03-02
Higher Expression Levels of Circulating miR-21, miR-19a and miR-10b Are Associated with High Risk Features in Breast Cancer.

Anfossi S, Giordano A, Cohen EN, Gao H, Cristofanilli M, Valero V, Alvarez RH, Hortobagyi GN, Woodward W, Ueno NT, Lee B-N, Reuben JM. The University of Texas MD Anderson Cancer Center, Houston, TX; Fox Chase Cancer Center, Philadelphia, PA

Background
MicroRNAs (miRs) have oncogenic and tumor-suppressor functions. MiR-21, miR-19a and miR-10b are overexpressed in breast cancer and regulate tumor cell migration, invasion and angiogenesis. We assessed the levels of miR-21, -19a, and -10b in sera of breast cancer patients and their association with the stage, histological type, hormonal receptor (HR) status and HER2 amplification in the primary tumor. Since circulating tumor cells (CTC) detected by CellSearch® are an independent and strong predictor of overall survival in metastatic breast cancer (MBC), we assessed the relationship between circulating miRs and CTCs.

Methods
The study consisted of 30 healthy donors (HD) and 95 breast cancer (BC) patients. Patients’ sera were collected before starting a new line of treatment. Total RNA was isolated, reverse transcribed to cDNA and then subjected to qRT-PCR for the detection of miR-21, -19a, -10b and -192 using the TaqMan MicroRNA Assay (Applied Biosystems, Foster City, CA). MiR-192 was used to normalize the expression levels of the other miRs. Fold-changes in expression of miRs were calculated using the 2^(-ΔΔCt) method, where DCt = mean CT_{mir-target} - mean CT_{mir-192}. CTCs were enumerated using CellSearch™ (Veridex LLC, Warren, NJ). Mann-Whitney U test was used to determine differences in serum miR expression levels between patients and HD.

Results
Of the 95 BC patients, 39 were non-MBC and 56 MBC. Patients grouped according to the receptor expression by immunohistochemical staining consisted of 27 HR+HER2-; 30 HR+HER2+; 20 HR-HER2-, and 18 HR-HER2-triple negative BC (TNBC). MiR-21 and miR-19a were higher in non-MBC patients than in HD (p=0.001; p=0.001, respectively). MiR-21, miR-19a and miR-10b levels were higher in metastatic patients than in HD (p<0.001; p<0.001; p=0.038, respectively). MBC patients had a higher median level of miR-21 than that of non-MBC patients (p=0.02). Patients with (HR+HER2+, HR-HER2-, TNBC) had significantly higher median levels of both miR-21 (p=0.018; p=0.009; p=0.045) and miR-10b (p=0.011; p=0.014; p=0.03) compared with HR-HER2 BC. HER2+ patients had higher median levels of both miR-21 and miR-10b than those of HER2- BC (p=0.033; p=0.01) and HD (p<0.001; p<0.009). Further, median miR-19a expression was higher in IBC patients than in non-IBC patients (p=0.025). Finally, patients with <5 CTCs had a higher median expression level of miR-10b than that of patients with ≥5 CTCs (p=0.042).

Discussion
High expression levels of miR-21, miR-19a and miR-10b in sera are observed in breast cancer patients, especially with advanced disease. HER2+ BC patients had higher serum levels of miR-21 and miR-10b than HER2-. IBC patients had a higher serum level of miR-19a than non-IBC patients. Moreover, patients with <5 CTCs had high serum levels of miR-10b that can be induced by Twist1 during the epithelial-mesenchymal transition (EMT) and, in part, explain the inability of CellSearch® to detect CTCs undergoing EMT.

P3-03-03
Congruence between Patterns of microRNA Expression and Histologic Grading of Invasive Breast Carcinomas.

Ellsworth DL, Croft DT, Field LA, Deyarmin B, Kane J, Ellsworth RE, Hooke JA, Shriver CD. Windber Research Institute, Windber, PA; Henry M Jackson Foundation, Rockville, MD; Walter Reed Army Medical Center, Washington, DC

Background: Histologic grading may be used as an indicator of prognosis in breast cancer; patients with low-grade carcinomas have ~85% ten-year survival compared to just 45% survival in patients with high-grade disease. Although useful for risk stratification, assigning nuclear grade is subjective, and a large proportion of carcinomas are classified as intermediate-grade with uncertain prognosis, thus limiting clinical utility. MicroRNAs (miRNAs) regulate gene expression and serve an important role in breast cancer development. In this study we examined miRNA expression profiles in low-grade and high-grade breast carcinomas to determine if miRNA expression is associated with pathological classifications of tumor grade.

Methods: Breast tumors were obtained from 69 patients enrolled in the Clinical Breast Care Project. Samples were partitioned into low-grade (n=30) or high-grade (n=39) categories using the Nottingham Histologic Score. Following laser microdissection of frozen tissue sections, miRNA was isolated from pure populations of breast tumor cells and hybridized to Affymetrix GeneChip® miRNA arrays containing over 800 human miRNA probes. Expression profiles were analyzed with Partek Genomics Suite using the miRNA Expression Module.

Results: We identified 30 unique miRNAs that showed differential expression at a False Discovery Rate (FDR) p<0.05 between low-grade and high-grade breast carcinomas. Gene targets for these miRNAs function primarily in metabolism and cell communication. Expression of hsa-mir-18a and hsa-mir-572 was significantly different between histologic grades at an FDR p<1x10^-5 and hierarchical clustering based on these miRNAs correctly classified 97% (29/30) of low-grade and 90% (35/39) of high-grade tumors. MiR-18a has been shown to inhibit ER signaling and promote cellular differentiation, while the role of miR-572 in breast carcinogenesis is not well known.

Conclusions: Dysregulation of miRNAs may accompany changes in cellular morphology typically used in histologic classification of breast carcinomas. Patterns of miRNA expression may improve reproducibility and clinical utility of tumor grading and may prove useful for prediction of recurrence and survival for patients with intermediate-grade carcinomas.
P3-03-04
Targeted Modulation of HER Receptor Signaling in Breast Cancer Using miR-21.
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Introduction: MicroRNAs (miRNAs) are multifunctional nucleic acids that regulate the stability and translational efficiency of targeted messenger RNAs. Endogenously altered miRNA levels have been linked in breast cancer to acquired chemo resistance and changed radiosensitivity. Thus the modification of miRNA expression represents an attractive therapeutic strategy. We now show that the targeted inhibition of miR-21 can modify expression of the HER2 receptor in breast cancer.

Materials and Methods: Using TaqMan-based technology the expression of five selected miRNAs (miR-7b, miR-10a, miR-17-5p, miR-125a, and miR-21) was investigated in formalin-fixed, paraffin-embedded breast carcinoma tissues expressing different levels of HER2 (n=60). Possible regulation of HER2 was explored in cell lines using lentivirus to stably downregulate or upregulate HER2 and miR-21 expressions. The effects were analyzed using qRT-PCR, Western blots, proliferation and migration assays.

Results: In tumor tissues we identified overexpression of miR-10a and miR-21, and underexpression of miR-17-5p and miR-125a in most of the tumor tissue samples. Significant associations (p≤0.05) were found between HER receptor expression and the expression of miR-17-5p and miR-125a, and miR-21 showed correlation with expression of ESR1 and PR. Furthermore, analysis of miR-21 downregulation in breast cancer cell lines showed inhibition of cellular proliferation and HER2 protein synthesis. Additionally, downregulation of HER2 expression decreased miR-21 expression and showed moderate change in cellular proliferation activity.

Conclusions: Based on these results we conclude that there is tight interplay between HER2 and miR-21 expression in breast cancer cells. Because miRNAs modulate gene expression we suggest that modulation of miR-21 has the potential to impact tumor therapy by interference with specific signaling pathways.

P3-03-05
Identification of miRNAs and Their Associated Target Genes Involved in Endocrine Resistance in Breast Cancer.
Healy NA, Creighton CJ, Fu X, Tsimelzon A, Hilsenbeck SG, Miller N, Kerin MJ, Osborne CK, Schiff R. Baylor College of Medicine, Houston, TX; National University of Ireland, Galway, Ireland

Background: Despite the widespread use of the selective estrogen receptor modulator tamoxifen or strategies of estrogen deprivation in the treatment of estrogen receptor-positive breast cancer, tumor resistance to endocrine therapy remains a serious clinical problem. MiRNAs are a class of endogenous, single stranded RNA molecules that play a pivotal role in the regulation of gene expression. Altered expression levels of these molecules have been implicated in a number of malignancies, including breast cancer. The aim of this study was to identify those miRNAs that may be associated with endocrine resistance and to further investigate their role in development of this resistance.

Methods: MCF7L breast cancer cells were cultured in regular parental medium (Par) or in phenol-red free, 10% charcoal-stripped FBS medium treated by estrogen deprivation (ED, >6months), tamoxifen (Tam, 10⁻⁸M, >6months), or estrogen (E2, 10⁻⁸M, >1month). Cells treated by long-term Tam or ED developed resistance (TamR or EDR) and resumed cell growth, which was confirmed by growth curve assay. Parallel short-term treatment (10 days) with estrogen deprivation or tamoxifen to represent the endocrine-sensitive phase, and short-term E2 treated cells as a control was also performed. A whole-transcriptome miRNA microarray was performed by High Throughput Genomics (Tucson, AZ), using cell lysates from Par, short-term/endocrine sensitive, E2 treated cells, TamR and EDR. The expression levels of several miRNAs were validated by real-time quantitative PCR (Q-PCR). MiRNAs dysregulated in TamR cells were integrated with predicted gene targets showing anti-correlated expression patterns across human breast tumors in The Cancer Genome Atlas (TCGA) datasets.

Results: The miRNA microarray identified 23 miRNAs that are upregulated in TamR cells (>1.7 fold change, p<0.005) compared to parental cells grown in the presence of E2, and 37 miRNAs that were downregulated (>50%, p<0.005). In addition, 6 miRNAs were observed to be upregulated in EDR and 3 downregulated in EDR compared with controls. Validation of four of these upregulated miRNAs within the TamR group, miR221, miR-222, miR-301b and miR-181c, was performed using Q-PCR. Previously identified genes associated with TamR were scanned for potential binding sites of these deregulated miRNAs using miRanda and TargetScan and many potential miRNA targeted genes are downregulated in TamR. Both miR-221 and miR-181c are predicted to target estrogen receptor gene (ESR1), and downregulation of this target gene was confirmed at the mRNA level by Q-PCR. A strong negative correlation was identified between ESR1 and both miR-181c and miR-221 expression levels in breast tumor samples, according to TCGA data. Work is ongoing to identify the role of miR-181c and other miRNAs in tamoxifen resistance, using a loss-of-function approach. Parallel miRNA profiling of multiple endocrine models is underway to identify commonly deregulated miRNAs associated with endocrine resistance.

Conclusions: This study has identified those miRNAs associated with endocrine resistance in breast cancer. These miRNAs may provide potential predictive markers of resistance to endocrine therapy and modulation of these may lead to increased therapeutic sensitivity.
hGH expression in human breast cancer have demonstrated that hGH expression is positively correlated with lymph node metastasis, tumour stage, HER-2 status and proliferative index. In mammary carcinoma cells autocrine hGH promotes cell proliferation, survival, migration/invasion and epithelial-to-mesenchymal transition as well as tumour formation in a xenograft model. In the current study we demonstrate that autocrine hGH regulates miRNA biogenesis in breast cancer cells.

Methods: Forced expression of hGH was established in the mammary carcinoma cell lines MCF-7 and T47D by stable transfection. miRNA expression was determined using miRNA microarray, quantitative real-time PCR (qPCR) arrays and qPCR assays. The expression levels of genes and proteins involved in miRNA biogenesis were assessed using real-time qPCR assays and Western blotting.

Results: More than 90% of miRNAs assayed were downregulated in hGH-transfected MCF-7 and T47D cells, compared to control transfected cells, as demonstrated by miRNA microarray and qPCR array. Changes in miRNA expression determined by microarray and qPCR array were verified using miRNA-specific qPCR assays. In MCF-7 cells, autocrine hGH did not significantly affect the mRNA expression levels of miRNA machinery components Drosha, DGC8R, PACT, TARBP, EXP-5, Dicer and AGO2 when compared to control transfected cells. Whereas in T47D cells autocrine hGH increased mRNA expression of TARBP, PACT and EXP-5 by 2.0, 2.3 and 1.8 fold respectively, compared to control transfected cells. Western blot analysis demonstrated that, autocrine hGH decreased protein levels of Dicer in MCF-7 cells, whereas Drosha and AGO2 were unchanged compared to control transfected cells. In T47D cells autocrine hGH decreased protein levels of Dicer, Drosha and AGO2 when compared to control transfected cells.

Conclusion: Our findings demonstrate that autocrine hGH stimulates global downregulation of miRNA expression in breast cancer cells. This may be one mechanism whereby autocrine hGH promotes tumour progression. Our results also indicate that autocrine hGH-mediated global downregulation of miRNA expression may occur through regulation of proteins involved in the miRNA biogenesis.

P3-03-07
A Novel Approach Integrating microRNA and mRNA Signatures of HMAPK Signaling Is Highly Predictive of ER- Status and Outcome in Breast Cancer – Role of HMAPK microRNAs in Repression of ER and p27.
Miller P., Clarke J, Koru-Sengul T, Brinkman J, El-Ashry D. University of Miami, Miami, FL

Deregulation of the MAPK signaling pathway in breast cancer is known to facilitate the down-regulation of the estrogen receptor and to contribute to the aggressive nature of ER negative and triple negative breast cancers. We have identified a microRNA signature indicative of hyperactive MAPK (HMAPK) signaling, which complements a previously established hyperactive MAPK gene expression signature. We have shown that hMAPK signaling also alters the regulatory activity of many microRNAs, including particular microRNAs with established roles in the biology of breast cancer, miR-221/222 and miR-22. Expression correlation with both the hMAPK microRNA signature and HMAPK mRNA signature is significantly associated with ER-negative status, increased tumor grade, high proliferation rate, and, importantly, poor disease specific survival among breast cancer patients, regardless of ER status. The hMAPK microRNA signature contains 127 microRNAs, 47 up-regulated and 70 down-regulated. Of note, hMAPK up-regulated microRNAs include miR-221/222 and 22, both of which have been demonstrated to target ER while miR-221/222 targets the cell cycle regulatory protein p27. Down-regulated microRNAs include miR-375, which positively regulates ER expression by down-regulating expression of an ER repressor. miR-221/222 and miR-22 exhibit both enhanced expression and enhanced regulatory activity in the context of HMAPK signaling, indicating an important role for these microRNAs in the biology of HMAPK signaling in breast cancer. In addition to these microRNAs, an unbiased approach of determining MAPK regulated microRNAs targeting the 3' UTRs of both ER and p27 will identify novel microRNAs involved in the MAPK regulated repression of ER and p27. These data not only suggest a regulatory role for microRNAs whose expression and biological activity are altered under conditions of hyperactivation of MAPK signaling in establishing and maintaining ER negativity and tumor aggression, but also indicate that hMAPK signaling may represent a novel aggressive tumor biology that is indicative of poor disease outcome in breast cancer.

P3-03-08
microRNAs in Mammary Stem-Like Cells and Triple-Negative Breast Cancer; Conserved Functions and Treatment Potential.
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Background: Mammary stem and progenitor cells are thought to be precursors of different subclasses of breast cancer. Triple-negative breast cancer exhibit similarities to stem or progenitor-like cells. microRNAs (miRNAs) play pivotal roles in both stem cell biology and oncogenesis. Given their potential as therapeutic targets, little is known, however, regarding miRNAs expression and functions in normal and breast cancer cells. Our objective was to identify which miRNAs are involved in mammary stem cell maintenance and differentiation and study their potential roles in triple-negative breast cancer. We investigated their expression during induced differentiation of mammary stem-like cells, correlated their expression to that of triple-negative breast cancer cells and studied the functions of the most regulated and conserved candidates.

Material and Methods: Using large-scale profiling and extensive qPCR confirmation we defined miRNAs regulated during stem-like cell differentiation. We investigated their expression in different breast cancer cell lines, and performed functional studies using miRNA mimics and inhibitors in normal stem-like cells and triple-negative breast cancer cells.

Results: Twenty-one miRNAs were strongly regulated in repeated rounds of mammary cell differentiation. The majority, including the miR-200 family and known tumor suppressor miRNAs, was upregulated during differentiation. Only 4 miRNAs, including the oncomiR, miR-17, were upregulated in the stem cell-like stage. Pathway analysis indicated complex interactions between regulated miRNA clusters and major pathways regulated during this transition, including stem cell maintenance, proliferation and differentiation. The cell model used exhibited gene expression profiles resembling basal-like poor prognosis breast cancer. Moreover, we present data of miRNAs that target the genes and networks responsible for this correlation. miRNAs detected in this study can also differentiate between human non-cancer and breast cancer cell lines, and a subclass of miRNAs, was specifically downregulated in a basal-like breast cancer cell line. The functions of five miRNAs (miR-200a, -200b, -146b, -148a and -206) were further investigated.

Discussion: Our findings suggest that the identified miRNAs have significant roles in biological pathways and networks associated with mammary stem/progenitor cell maintenance and triple-negative breast cancer.
African breast cancer is biologically distinct and shows remarkable
differences in histological type, grade, hormone receptors & HER2
status when compared with breast cancer in white women. The early
age at presentation, predominance of high grade and triple negative,
but not necessarily basal phenotype, may explain the poor prognosis
and requires tailoring treatment strategies to target this unique profile.

**P3-04-02**

**Bevacizumab Treatment Alters Intrinsic Subtypes in a VEGF-
Reinforced Xenograft Model of ER-Positive Breast Cancer.**

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U, Mehta R, Li L, Ivan M, Badve S, Sledge GW. Indiana University
School of Medicine, Indianapolis, IN

Background: Anti-vascular endothelial growth factor (anti-VEGF)
therapy improves disease-free but not overall survival in metastatic
breast cancer. To seek further insight on resistance to anti-VEGF
antibody bevacizumab (BEV) at the molecular level, we developed
cancer xenograft models allowing comparison of tumor
response at different time-points. Here we report the gene expression
and miRNA analyses of response and non-response to BEV in these
models.

Methods: MCF-7 cells transfected with control vector (ML20) or
VEGF (MV165) were implanted into the mammary fat pads of
athymic mice. Tumors from short-term treatment with BEV (3
weeks; Responders to BEV, R) or long-term treatment (8 weeks;
Non-Responders, NR) or with vehicle control group (V) were subjected
to whole-genome gene expression analysis (Human WG-6v2 Expression
Beadchips, Illumina) and miRNA profiling (TaqMan ArrayHuman
MicroRNA A+B Cards Set v3.0, Applied Biosystems). Validation of
the chosen genes was performed using quantitative real-time RT-PCR
(qRT-PCR) and Immunohistochemistry (IHC).

Results: Short-term treatment to BEV (3 weeks; 5 mg/kg, i.p./twice
weekly) inhibited primary tumor growth significantly in MV165
xenografts compared with vehicle control, whereas BEV treatment
did not affect the tumor growth in the ML20 model. MV165 xenografts
progressed after 8 weeks of BEV treatment. Gene set enrichment
analysis (GSEA) revealed that luminal A-related gene sets were
enriched in MV165-R compared to MV165-NR group including
DESMEDT (ESR1), SMID_Breast_Cancer_Luminal_A_up, and
MASSARWEH_Tamoxifen_Resistance_Down. Myoepithelial-
specific gene sets were upregulated in both the R and NR groups
compared with the vehicle group. qRT-PCR analysis showed that
estrogen receptor alpha (ESR1) representative for luminal A
decreased significantly in the MV165-NR group (P=0.001) compared to vehicle.
In contrast, Cytokeratin 5 (KRT5) levels increased significantly in both
R (P=0.02) and NR (P=0.03) groups. In addition, KRT14 was
upregulated in R (P=0.04) and in NR (P=0.14) group in comparison
with the vehicle group, suggesting the upregulation of myoepithelial
phenotype specific to BEV treated MV165 model, but not ML20
model. Similar results were obtained by IHC. Consistent with mRNA
changes, ESR1 regulated miRNA such as miR-107 (P=0.007) and
miRNA important in tamoxifen resistance such as mir-451 (P=0.0003)
were also altered in MV165-NR group compared to vehicle.
Conclusion: These results suggest that treatment with BEV may alter
the intrinsic subtypes in the presence of VEGF expression. These data
may help to explain the variable results to anti-VEGF therapy based
on the duration of BEV treatment.

**P3-04-01**

**Molecular Characterization of African Breast Cancer; Results
from a Large Tissue Microarray Study.**

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**Introduction**

Breast cancer in African women has been understudied for decades.
Evidence derived mainly from studies on African-American women,
supports that tumours of black women are biologically different and
more aggressive than those occurring in the white population. Of the
4 main taxonomic groups of breast cancer, basal tumours are more
represented in those women. In the present state of knowledge, very
little is known about the biology and molecular profile of breast cancer
in Africa. The aim of this study was to test the hypothesis that the
molecular profile of African breast cancer is distinct from its Western
counterpart. This was achieved by collecting a large cohort of breast
carcinomas from an indigenous African population for phenotypical
characterization and testing for expression of potential predictive
and prognostic markers.

**Methods**

Breast tumours were collected via collaboration with five centres
in Nigeria (the most populous country in Africa) and assembled
into tissue microarrays (TMAs). All tumours were reviewed by a
specialist breast pathologist following the Royal College of
Pathologists (RCPath) guidelines to confirm diagnosis, type, grade
and nodal status. Patients age, tumour size and clinical data, where
available, were collected from the original pathology reports and
case notes. Representative tumour areas were selected and marked
for TMA construction. TMA sections were stained for a range of
markers including hormone receptors (ERα, ERβ, PR, AR), cyclin
D, HER2, Ki67, bcI2, basal (CK5/6, CK14) and luminal cytokeratins
(CK18, 19).

**Results**

A total of 830 tumours were assembled into TMAs. The mean age
diagnosis was 47.69yrs with 58% of patients presenting under the
age of 50. Only 8.5% of tumours were of grade 1. Most tumours
(87%) were of ductal no special type, followed by lobular and
metaplastic carcinomas. The majority of the tumours were ERα, PR
and HER2 negative (77%, 80% and 81% respectively). The triple
negative tumours were the predominant phenotype (55.6%). Luminal
A type tumours comprised 24.3% followed by the HER2 positive
(87%) were of ductal no special type, followed by lobular and
metaplastic carcinomas. The majority of the tumours were ERα, PR
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**P3-04-01**

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differences in histological type, grade, hormone receptors & HER2
negative tumours were the predominant phenotype (55.6%). Luminal
A type tumours comprised 24.3% followed by the HER2 positive
(13.9%) and luminal B tumours (6.2%). The differences of all those
parameters were statistically highly significant (p<0.001). Most
tumours expressed ERβ including 75% of those that were ERα/PR
negative. A large proportion of the tumours (22%) were of the basal
phenotype of which two thirds were also triple negative. Over half
of the triple negative tumours were also node positive.

Hierarchal cluster analysis showed the basal tumours dendrogram
to comprise two groups; one showing clustering of ERα/PR/HER2 and
the second showing clustering of ERβ with CK5 and CK14.

**Conclusion**

To our knowledge, this is largest and most comprehensive study of
African breast cancer to date. Our data confirms the hypothesis that
African breast cancer is biologically distinct and shows remarkable
cancer and support their further evaluation as potential prognostic
markers and therapeutic targets in the treatment of breast cancer.
Identification of Hormone-Responsive Genes as Biomarkers for Menstrual Cycle Phases and Menopausal Status.

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Background: The definition and validation of biomarkers in archived breast tissue samples for prognostic research is limited by the fact that the exact menstrual cycle phases and menopausal status at the time of tissue sampling is often unknown or not accurate by patient survey. Biomarkers that vary with menstrual cycle phases in premenopausal women would be difficult to standardize. There are also significant differences in gene expression in pre- and post-menopausal women. Therefore, menstrual cycle fluctuation and menopausal status need to be considered for all candidate biomarkers as part of the validation process. The aim of this study is to identify genes responsive to different hormones to accurately define menstrual cycle phases and menopausal status.

Methods: We studied gene expression profiles of 18 random fine-needle aspirate (rFNA) samples from unaffected contralateral breast (8 pre-menopausal, mean age 44.5; 10 post-menopausal, mean age 58.8) and investigated the correlation between gene expression and serum hormone levels of estradiol (E2), progesterone (P4) and follicle stimulating hormone (FSH). Genes that were highly correlated with the serum levels of each hormone (Pearson correlation coefficient r > 0.60) were considered as specific hormone-responsive genes (P < 0.0085). The combined gene profiles of hormone-responsive genes were used to dissect samples in different menstrual cycle phases and menopausal status. Selected genes related to mammary gland development and hormone regulation based on gene function and gene network analysis were validated using qRT-PCR in 18 original rFNA samples and in 28 independent samples.

Results: From 35,964 genes and 12,838 undesignated transcripts, we identified genes/transcripts highly correlated with E2 (1091 genes), P4 (127 genes) or FSH (58 genes). The most significantly correlated genes in each group were selected to define four panels of genes: Panel A-21 genes stimulated by E2 (r > 0.78); Panel B-22 genes stimulated by P4 (r > 0.75); Panel C-7 genes stimulated by FSH (r > 0.65); and Panel D-10 genes suppressed by FSH (r < -0.65). Hierarchical clustering analysis using the combination of gene panels dissected the samples into four clusters based on three phases of menstrual cycles and post-menopausal status. Specifically, high panel C and low panel D expression segregated post- from pre-menopausal samples. Low expression of panel A and B genes dissected early follicular phase from late follicular and luteal phases, while higher expression of panel B genes discriminated luteal phase from late follicular samples.

Conclusion: Our results indicate that the menstrual cycle phases and menopausal status determined by age, patient survey and serum hormone concentrations are reflected in the expression of specific gene sets in the normal breast. The combination of hormone-responsive gene panels would allow the classification of breast samples regarding to the menstrual phases and menopausal status at the time of sampling. It would also facilitate the selection and validation of breast cancer biomarkers that are independent of menstrual cycle fluctuation and menopausal variation for clinical use.

Patterns of Distant Metastasis According to the Molecular Subtypes of Breast Cancer; Results of 529 Breast Cancer Patients.

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Background: Distant metastasis from breast cancer arises from various sites. But few studies concerning factors that can predict metastasis patterns in breast cancer has been reported. In this study, we analyzed the effect of breast cancer molecular subtypes on distant metastasis patterns and tried to determine factors that predict metastasis sites.

Patients and methods: From January 1995 to January 2004 at Yeungnam university hospital, patients diagnosed with the primary invasive breast cancer and received treatments were included in this study. Patients with bilateral breast cancer or distant metastasis at diagnosis were excluded. After analyzing estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor 2 (HER2), epidermal growth factor (EGFR) and cytokertin (CK) 5/6 status, we classified patients into 5 categories, luminal A, luminal B, HER2-enriched, basal-like, and normal (triple negative nonbasal) breast cancers. Distant metastatic patterns of each category were analyzed.

Results: 529 patients were eligible for tissue microarray analysis and median follow-up period was 7.7 years. In this period, total 82 patients (15.5%) had locoregional relapse or distant metastasis and distant metastasis were identified in 54 patients (10.2%). Each distant metastatic rate was 10.5% (33/313) in luminal A, 8.7% (4/46) in luminal B, 10.2% (6/59) in HER2-enriched, 7.9% (7/89) in basal-like, and 18.2% (4/22) in normal subtype. Most frequent site of distant metastasis in all patients was bone and such result was consistent with Luminal A subtype. Liver metastasis was most frequent in Luminal B subtype, lung metastasis in HER2 enriched and normal subtype and brain metastasis in basal-like subtype.

Conclusions: There was a definite association between breast cancer molecular subtype and distant metastatic pattern. If more patients and prolonged follow up periods are analyzed, we would be able to determine the best follow up intervals, methods and treatment directions concerning subtypes of breast cancer.

Expression Profiles of RANK and RANKL mRNA and Protein in the Mammary Gland of Female Cynomolgus Monkeys after Long-Term Treatment with Different Menopausal Hormone Therapies.

Branstetter D, Wood CE, Rohrbach K, Borgerink H, Dougall WC. Amgen Inc., Seattle, CA; Wake Forest School of Medicine, Winston-Salem, NC

Background: RANK and its ligand (RANKL), key factors for bone remodeling and metastasis, are crucial for the development of the mouse mammary gland during pregnancy. OPG also binds RANKL and inhibits RANKL action. Treatment of mice with progesterone increases RANKL expression in mammary epithelium. RANKL functions as a major paracrine effector of both progesterone-dependent...
mitogenic action in mammary tissue, and also in ovarian hormone-dependent expansion and regenerative potential of mammary stem cells. RANKL inhibition in vivo reduces progesterin-induced mammary preneoplasias and adenocarcinomas. In postmenopausal women, the addition of a progesterin to estrogen therapy has been associated with significant increases in mammary proliferation, breast density, and breast cancer risk and recurrence. The goal of this study was to characterize the expression of RANK, RANKL, and OPG in the mammary gland following estrogen + progesterin therapy in comparison with estrogen-alone therapy or placebo in a postmenopausal primate model.

Methods: Ovariectomized female cynomolgus monkeys (Macaca fascicularis) were randomized into 3 treatment groups: placebo, oral conjugated equine estrogens at 0.625 mg/d (CEE) and CEE with medroxyprogesterone acetate (MPA) at 2.5 mg/d (CEE+MPA). Treatments were administered in the diet for 2 years. RANK, RANKL, and OPG mRNA were measured in mammary gland samples (n = 28 to 31/group) using quantitative real-time reverse transcriptase polymerase chain reaction (qPCR). Antibodies against human RANK (N-2B10 and N-188; Amgen) and human RANKL (M366; Amgen) were used for immunohistochemistry (IHC) of formalin-fixed tissues (n = 20 to 23/group) along with an isotype control. The intensity of IHC staining was scored on a semi-quantitative scale (0 = absent, 1 = weak, 2 = moderate, 3 = intense). Incidence was scored as a positive IHC signal (any intensity).

Results: Analysis of mRNA revealed that treatment with CEE+MPA resulted in a significantly greater ratio of RANKL to OPG compared to treatment with placebo (P < 0.01) or CEE (P < 0.05). IHC demonstrated RANK protein was predominately localized to basal cells of ducts and acinar cells, while RANKL protein was localized to luminal cells. RANK protein was detected in mammary glands of animals in all groups: placebo (74%), CEE (55%), and CEE+MPA (53%). RANKL protein was expressed in the animals treated with CEE+MPA (74%) and CEE (15%), but not in placebo-treated animals. Quantitative expression of specific mRNA markers for epithelial proliferation (MKI67) and density (KRT19) was highest for CEE+MPA, intermediate for CEE, and lowest for placebo.

Discussion: Key components of the RANKL/RANK pathway are expressed in the normal postmenopausal primate mammary gland, modulated by long-term estrogen+progesterin exposure at clinically relevant doses, and associated with changes in estrogen+progesterin-driven proliferation of mammary tissue.

P3-04-06
Comparison of MammaPrint, BluePrint, and TargetPrint with Clinical Parameters in Patients with Breast Cancer: Findings from a Prospective United States Cohort.
Nguyen B, Sinha R, Kerlin D, Barone J, Garcia A, Yao K, Rivera E, Stork-Sloots L, Deck K. Long Beach Memorial Health Care, Long Beach, CA; Rockwood Clinic, Spokane, WA; John Muir, Walnut Creek, CA; Comprehensive Breast Care of San Diego and Sharp Memorial Hospital, San Diego, CA; University of Southern California, Los Angeles, CA; North Shore University Health System, Chicago, IL; The Methodist Hospital/Weill Cornell University, Houston, TX; Agenda Inc, Irvine, CA; Saddleback Memorial Medical Center; Laguna Hills, CA

Background: MammaPrint (MP) is a powerful predictor of disease outcome in early stage breast cancer. In addition, TargetPrint (TP), a quality approved microarray-based test that measures the mRNA expression level of ER, PR and HER2 and an 80 gene expression Molecular Subtyping profile BluePrint (BP) were developed. In the present study, MP, BP and TP were measured in a prospective U.S. breast cancer patient cohort.

Methods: MP results were evaluated in fresh tumor samples from 141 breast cancer patients (T1-4N0-2; median age 62 [35-97 yr]) collected by core needle biopsy or from a surgical specimen between July 2008 and February 2011. We compared treatment advice as recommended by NCCN guidelines and classification according to MP. In addition, we compared IHC/FISH ER, PR and HER2 assessments with TP. The MP and BP results were used to subtype the patients into molecular subgroups.

Results: For the group of patients (n=69) for which NCCN recommends the use of a multi-gene signature for determining chemotherapy treatment recommendations, 50 patients were classified as High Risk and 19 as Low Risk by MP. Comparison of TP with IHC/FISH indicated a concordance of 98% for ER, 91% for PR, and 94% for HER2. For a subgroup of 63 patients combined MP and BP results were available: 22 patients were Luminal-type/MP Low Risk, 31 patients were Luminal-type/MP High risk, 1 patient was Her2-type/MP Low Risk, 2 patients were Her2-type/MP High Risk and 7 patients were Basal-type/MP High Risk.

Conclusion: The multi-gene signature MammaPrint, as well as BluePrint and TargetPrint provides additional information for treatment guidance. By combining MammaPrint with the BluePrint molecular subtyping profile, specific groups of patients can be recognized that are at high risk of recurrence and that would possibly benefit from specific treatment. This study shows that TargetPrint provides high quality second opinion for local IHC/FISH assessment.

P3-04-07
Physiological Concentrations of Genistein and 17β-Estradiol Inhibit MDA-MB-231 Breast Cancer Cell Proliferation by Increasing Bax/Bcl2 Ratio and Decreasing pERK1/2 Expression.
Rajah TT, Peine KJ, Du N, Serret CA. DePaul University, Chicago, IL

Background: Our previous results were the first to show that physiological concentrations (1 µM) of genistein, a soy component, in the presence of 17β-estradiol (1 nM) inhibited the cell proliferation of MDA-MB-231 (Estrogen receptor (ER) β positive) breast cancer cells. These results are relevant in premenopausal women with breast cancer of the ERα-negative and ERβ-positive type, especially given the increasing trends of soy intake among the US population in the past decade. The aim of the present study was to identify the mechanism by which genistein plus 17β-estradiol inhibits the cell proliferation of ERα-negative and ERβ-positive breast cancer cells. Our hypothesis is that the balance of signaling actions by genistein plus 17β-estradiol from different signaling pathways is likely to lead to the cell’s choice to either proliferate or enter the apoptotic pathway. For this purpose, the effect of low and high concentrations of genistein (1 µM and 100 µM) in the presence or absence of 17β-estradiol (1 nM) was studied on the expression of cell signaling proteins involved in cell proliferation, survival and apoptosis (pERK1/2, pAkt, Bax and Bel2) and correlated to cell proliferation and apoptosis in MDA-MB-231 (ERβ positive and ERα negative) breast cancer cells.

Methods: Cell proliferation was determined by the MTT assay, apoptosis determined microscopically by the use of acridine orange and ethidium bromide dyes and the expression of cell signaling proteins by western blotting.
Results: Our results show that 1µM genistein plus 17β-estradiol significantly increased apoptosis (p<0.05) as compared to the control (12.47% vs 5.87% respectively). Increased expression of Bax/Bcl2 (2.5 fold) along with a decreased expression of phosphorylated ERK1/2 (2 fold) was observed in cells treated with 1µM genistein plus 17β-estradiol as compared to the control cells. Phosphorylated Akt did not show any differences in the treatment condition as compared to the control. High concentrations of genistein (100 µM) in the presence or absence of 17β-estradiol also increased apoptosis; however these changes could not be correlated to the expression of Bax/Bcl2, or pERK1/2.

Conclusion: In conclusion, our results show that physiological concentrations of genistein in the presence of 17β-estradiol inhibit cell growth through apoptosis via increased Bax/Bcl2 and a concomitant decrease in pERK1/2 expression. Our results also suggest that different concentrations of genistein elicit cell responses through different signaling mechanisms. These results are especially relevant to the cohort of premenopausal women with breast cancer of the ERβ positive and ERα negative type.

P3-05-01


Background:
Breast cancer is currently classified in 3 groups based on estrogen receptor alpha (ER) and human epidermal growth factor receptor 2 (HER2/ERBB2) gene expression: one basal-like (ER-ERBB2-), one HER2-enriched (ERBB2+) and one luminal (ER+). Yet, in transcriptome-based classifications, ER-ERBB2+ group partially overlaps with more recently defined ER-AR+ (androgen receptor positive) group. This type was named molecular apocrine, in reference to the histopathologically characterized apocrine carcinomas (H-Apo), in which a marked activation of AR signaling was demonstrated with a distinct proteomic signature. H-Apo tumors correspond to 1% of invasive breast carcinomas and are clearly morphologically distinct from other AR+ tumors. However, no specific H-Apo transcriptome signature has been reported for this sub-group. In an effort to better characterize those tumors, we have performed a meta-analysis of genomic data, focusing on the ER-AR+ breast subset.

Samples and Methods:
Chips were from Affymetrix array generations HG-U133. 258 profiles were unpublished and 1145 were from published or in press data. Gene expression was carried out after GC-RMA normalization. Unsupervised hierarchical clustering and other statistical analysis were performed with R software.

Results:
160 of the 1403 investigated tumors were ER-AR+. An unsupervised hierarchical clustering clearly identified a small subgroup of 14 closely tumors expressing high transcripts levels of PIP, HPGD, ACSM1, AR, SDR5A1, HS3DB1. This profile was very similar to the proteomic signature previously described for the H-Apo tumors. In addition, the pathology report, although available only for 4 of those 14 tumors, described them as typical apocrine carcinomas. Taken together, these data suggested that this cluster was the H-Apo subgroup. Unexpectedly, when using the transcriptomic PAM50 classification, 13 were classified as Luminal and only 1 as HER2-enriched, although the 14 tumors were all ER-negative. CGH analysis with Agilent 244K chips was carried out with 25 ER-AR+ tumors, of which 5 were H-Apo carcinomas. Importantly, those 5 H-Apo tumors exhibited fewer DNA lesions than the other ER-AR+ apocrine tumors (17% copy number alterations in H-Apo group versus 41%, p=0.02). More CGH data are currently under investigations and will be discussed.

Discussion:
The histopathologically characterized apocrine carcinomas (H-Apo) display transcriptomic signs of active androgen metabolism and fewer DNA lesions than other molecular apocrine tumors. This could suggest that molecular apocrine and H-apocrine tumor derive from the same cell of origin, but that only H-Apo retains morphological apocrine features, possibly due to the presence of fewer genetic lesions. In any case, the prominent androgen signaling activation warrants functional assays of anti-androgen in these breast cancer subtypes.

P3-05-02
Subtype-Specific Co-Occurrence of Atypical Hyperplasia and In Situ Carcinoma with Invasive Breast Cancers.

Kovatch AJ, Kvecher L, Chen Y, Bekhash A, Hooke JA, Shriver CD, Mural RJ, Hu H. Windber Research Institute, Windber, PA; Walter Reed Army Medical Center, Washington, DC; MDR Global Systems LLC, Windber, PA

Background: Atypical ductal hyperplasia (ADH), lobular carcinoma in situ (LCIS), and ductal carcinoma in situ (DCIS) are considered risk factors for the development of invasive breast cancer (IBC). The co-occurrence of these lesions with IBC may provide insights into cancer initiation and development. IBC subtypes have distinct clinicopathological features. A clinically practical IHC-based subtyping classification has been developed based on the expression of ER, PR, HER2, and Ki67, defining Luminal A (LA), Luminal B (LB), HER2+, and Triple Negative (TN) subtypes. The Walter Reed Army Medical Center (WRAMC), through the Clinical Breast Care Project (CBCP), has enrolled over 500 IBC subjects with single pathologist review and central lab analysis. The co-occurrence of ADH, LCIS, and DCIS will be studied in relation to IBC subtypes.

Methods: Subjects were enrolled following IRB-approved protocols. IBC patients enrolled at WRAMC were selected and their clinical and pathology data were reviewed. ER and PR positivity is defined as ≥ 15%. For IBC subtypes, LA is ER+/HER2-/Ki67-; LB is either ER+/HER2-/Ki67+ or ER+/HER2+; HER2+ is ER-/PR-/HER2+; TN is ER-/PR-/HER2-. Statistical analysis was performed using SAS, and the Chi-Square test was used for categorical data analysis supplemented by the Fisher’s Exact test where appropriate. For age analysis, ANOVA was performed with Bonferroni adjustment for multi-pair t-test.

Results: A total of 459 IBC patients were identified and categorized into LA (41.6%), LB (27.7%), HER2+ (10.2%), and TN (20.5%). Many of the previously reported subtype-specific characteristics were confirmed. Age at diagnosis varied by subtype (p=0.0034) with LA being the oldest (Mean±SD=59.2±11.6 years) and TN the youngest (Mean±SD=52.2±12.6 years, p=0.0048). Ethnicity distribution of African American (AA) relative to Caucasian American patients varied significantly in subtypes with AA=18% in LA, 31% in LB, 32% in Her2+, and 42% in TN (p=0.0008). The grade, the AJCC stage and its components T and N were all significantly different among the subtypes (p ranges
from <0.0001 to 0.0020). The grades and stages were consistently lowest for LA, highest for HER2+ and TN. We further found that the co-occurrence of ADH, DCIS, and LCIS with IBC were subtype-specific with the following distributions: ADH—LA (25.1%), LB (18.9%), HER2+ (0%), and TN (6.4%) (p<0.0001, n=78); DCIS—LA (63.4%), LB (76.4%), HER2+ (80.9%), and TN (58.5%) (p=0.0039, n=311); LCIS—LA (36.7%), LB (19.7%), HER2+ (4.3%), and TN (6.4%) (p<0.0001, n=103).

**Discussion:** By including Ki67 in IHC-based IBC subtyping we confirmed many subtype-specific clinico-pathological characteristics in the CBCP WRAMC population. We further report subtype-specific co-occurrences of ADH, DCIS, and LCIS. These co-occurrence patterns may reveal distinct developmental mechanisms between the different subtypes of IBC.

**P3-05-03**

**Transcriptomic Validation of Molecular Classification of Invasive Ductal Carcinoma Based on Immunohistochemical Markers and Grade.**


Transcriptomic analyses identified four major groups among invasive ductal carcinomas, associated with different clinical outcomes. Their definitions in practice is still matter of debate. Our aims were 1) to validate definitions based on immunohistochemical markers and grade: luminal A= ER and or PR+ve, grade I HER2-ve, luminal B= ER+ve and /or PR+ve, HER2+ve or grade III, HER2 enriched carcinomas= HER2+ve and ER+ve, basal-like/triple negative carcinomas= grade III, ER-ve, PR-ve, HER2+ve and expression of at least one of the basal-like markers (CK5/6, CK14, EGFR); 2) to refine this immunohistochemical definition.

142 consecutive tumors were selected in our tumour bank (42 triple-negative and grade III; 31 HER2+ve and ER-ve; 35 luminal B ER or PR+ve and grade III (7 cases) or HER2+ve (28 cases); luminal A (34 ER+ve or PR+ve, grade I)). Transcriptomic analyses were performed using Affymetrix U133+2 arrays (good quality RNAs obtained for all frozen specimens). The molecular classes determined according to proposed definitions were compared to those obtained with unsupervised clustering analyses using data after GC-RMA normalisation (with the intrinsic gene list genes and with the highest variance genes). Marker patterns of expression (CK 8/18, 5/6, 14, EGFR, BCL2 and Ki67) were analysed within each molecular class.

Based on the phenotypical definition and grade, 10 and 9% of cases were misclassified respectively using unsupervised clustering either with the intrinsic gene list or the highest variance genes. The misclassified tumors were luminal B HER2+ve cases with low ER level of expression, or HER2+ve and ER-ve cases classified among the triple-negative group. 39 out of 42 (93%) triple negative expressed at least one of the basal markers. RP expression pattern differed between luminal A and B carcinomas. All luminal A showed at least > 15% of positive cells and 65% of them harboured > 50% of positive cells. In contrast, luminal B showed < 15% of positive cells in 70% of the cases. Bel2 was negative in 40% of the luminal B cases and positive (> 20% stained cells) in more than 90% of the luminal A cases. CK14 was positive (i.e. > 1% positive cell) in 65% of the triple negative cases, compared to CK5/6 positive in 58% of the cases. Ki67 was > 20% in 90% of the triple negative and in 55% of luminal B cases compared to less than 5% of the luminal A cases. 20 and 30% of HER2+ve ER-ve carcinomas expressed CK5/6 , CK14 and EGFR associated in more than 90% of the cases to CK8/18 positivity (> 20% positive cells).

Identification of molecular classes of breast carcinomas was accurately determined by immunohistochemistry and grade. Low level of RP expression and BCL2 negativity were part of the luminal B phenotype. CK14 was more sensitive in this population of triple negative carcinomas to identify basal-like carcinomas. Ki67 was highly expressed in the vast majority if not all breast triple negative carcinomas. HER2+ve ER-ve carcinomas can express basal markers but in contrast to basal-like carcinomas, associated in the large majority of the cases to CK8/18 expression. The accurate determination of molecular groups of breast carcinomas should be a key parameter for development of targeted therapies within each group.

**P3-05-04**

**Changes in Recurrence Risk of Breast Cancer Intrinsic Subtypes over Time.**


**Background:** Gene expression profiling and their immunohistochemistry-based surrogates have consistently revealed prognostically significant breast cancer (BC) subtypes: Luminal A (Lum A), Luminal B (Lum B), HER2, Basal-like (BL) and Triple negative phenotype-nonbasal (TNP-nb). In addition, there are clinical evidence that hazard of BC recurrence varies over time with two peaks of high risk at 18-24 and 60 months. This study compares the time-related patterns of recurrence within BC subtypes.

Methods: Tissue microarrays were constructed from 937 early BC patients diagnosed and treated at our Hospital from 1982 to 2005 with available archival paraffin tissue blocks. BC subtypes were defined using an immunopanel of estrogen receptor, progesterone receptor, HER2, epidermal growth factor receptor, cytokeratin 5/6 and Ki67 by prespecified published methods. Univariate and multivariate analysis (Cox regression) were performed on progression-free survival. Smoothed curves for hazard rates (HR) were estimated by a Kernel-like smoothing procedure. The statistical analysis was done by using the R software environment.

Results: Cases were classified as follows: Lum A 46.8%, Lum B 25.2%, HER2 11.3%, BL 11.3%, TNP-nb 5.4%. None of the patients were treated with adjuvant trastuzumab. With a median follow up of 80 months age, tumor size, nodal status and intrinsic subtypes were independent prognostic factors. HER2 and BL show high and early peak in HR curves and decreasing sharply to 36 and 48 months respectively. HR in Lum A, Lum B and TNP-nb exhibit a smoother and nearly steady curve.

**Hazard Rates for BC Subtypes Recurrence**

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Lum A</th>
<th>Lum B</th>
<th>HER2</th>
<th>BL</th>
<th>TNP-nb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum HR (%)</td>
<td>8.6</td>
<td>8.7</td>
<td>8.3</td>
<td>7.6</td>
<td>3.6</td>
</tr>
<tr>
<td>Time of Maximum HR (months)</td>
<td>25</td>
<td>30</td>
<td>19.2</td>
<td>21.2</td>
<td>17.9</td>
</tr>
</tbody>
</table>

Conclusions: BC subtypes have distinct outcome but also displays different pattern of recurrence over time. These data might imply that pathways underlying early and late recurrences could be different. This additional information would suggest the convenience of considering different timings and duration of adjuvant treatments depending of BC subtypes, and also in the design of surveillance recommendations.
P3-05-05
Cyclin D1 Gene Amplification Is Rarely Heterogeneous in Breast Cancer.

Background: Amplification of Cyclin D1 (CCND1) occurs in about 10 - 20% of breast cancers and has been suggested to predict resistance to anti-hormonal therapy. As the diagnostic accuracy of predictive biomarkers can be substantially limited by regional expression differences within tumors, heterogeneity of CCND1 amplification was assessed in this study. To assess heterogeneity, a novel tissue microarray based analysis platform was developed.

Material and Methods: To comprehensively assess the three-dimensional molecular composition of breast cancers, a “heterogeneity TMA” was constructed containing 8 different tissue cylinders from as many different cancer containing tumor blocks as possible (at least 4) from 147 primary breast cancers. Additional tissue samples were taken from 1-4 corresponding nodal metastases from 35 of these patients. Dual labeling fluorescence in situ hybridization (FISH) with probes for CCND1 and centromere 11 was applied.

Results: The analysis revealed amplification in 29 of 133 (21.8%) patients with interpretable FISH data. CCND1 amplification was more frequently seen in ductal (22 of 87; 25.29%) than in lobular type (5 of 32; 15.63%) (p=0.251). CCND1 amplification was also associated with high tumor grade with amplification rates of 1 of 18 (5.56%) in grade 1, 15 of 72 (20.83%) in grade 2 and 12 of 40 (30%) in grade 3 carcinoma (p=0.075). CCND1 amplification was more frequently seen in ER positive cases (27 of 110; 24.55%) than in ER negative cases (1 of 17; 5.88%) (p=0.052). No association could be found between CCND1 amplification and tumor stage (p=0.445) and CCND1 amplification and PR status (p=0.752). Heterogeneous amplification status was detected in 9 of 29 (31.00%) amplified tumors, i.e. in 6.8% of all informative cases. Heterogeneity was successfully validated on large sections in all 4 heterogeneous cases with high level amplification. In the remaining 5 “heterogeneous cases” discordant results were due to variable interpretation of borderline amplification results with CCND1/centromer 11 ratios between 1.7 and 2.3. There were no discrepancies seen between primary tumors and matched lymph node metastases.

Discussion: The high degree of homogeneity seen for CCND1 amplification suggests that this alteration represents an early event in tumor development/progression in a subset of breast cancers. CCND1 status determined in a small biopsy will be highly representative of the entire tumor and will thus be appropriate for predicting treatment outcome.

P3-05-06
Progression of Breast Cancer Molecular Subtypes through Different Clinical Stages.
Ciriñelos EM, Castaneda CA, Andrés E, Gomez HL, Castellano D, Mendiola C, Manso L, Ghamem I, Farfan C, Cortes-Funes H. Hospital Universitario 12 de Octubre, Madrid, Spain; Instituto Nacional de Enfermedades Neoplásicas, Lima, Peru

Purpose: Molecular classification of breast cancer (BC) through immunohistochemistry classifies patients globally in 4 subtypes with similar features and prognosis. The present study was designed to compare features of BC subtypes in early versus locally advanced clinical stages. A secondary objective was compare molecular subtypes of the primary vs recurrences.

Methods: The study included 1621 patients with non-metastatic invasive BC that were consecutively diagnosed at Hospital Universitario 12 de Octubre, Madrid, between 1997 and 2007. Luminal A was defined as ER+ and PR+, HER2-negative and Ki67 < 14%. Luminal B was defined as PR-negative, HER2-positive or Ki67≥14%. HER2 was defined as ER and PR-negative, and HER2-positive. Triple negative (TN) were tumors with ER, PR and HER2 -negativity. Clinicopathological characteristics and outcomes were retrospectively reviewed. Variables were compared with the X2 test, and survival curves were evaluated with Kaplan-Meier method.

Results: Most patients were diagnosed as T1 (48%) and T2 (39.7%) clinical stages, and classified as Luminal B (49%) and Luminal A (29%). GIII frequency increased from T1 to T3-4 in Luminal A (p=0.002), and Luminal B (p=0.051) subtypes, but not in HER2 (p=0.867) or TN (p=0.53).

Median ER tended to decrease from T1 to T3-4 in Luminal B (p=0.062). Median Ki67 of T1 and T3-4 were not different in HER2 (p=0.867) and TN (p=0.533) subtypes.

Molecular subtypes carry significant different prognosis (DFS and OS) in all T stages except for T1a cases. When T3-4 and T1 stages of the same molecular subtype were compared, a significant shorter DFS were found for more advanced stages in the Luminal A (p=0.0002), Luminal B (0.0001) and TN (0.0017), but not for HER2 (p=0.54) subtype. And, similar results were found for OS.

We compared molecular subtypes in the primary tumor and in the metastatic site in 83 cases (excluding contralateral recurrences) and found change of phenotype in 54% of the cases. Changes fromLuminal A to a more aggressive phenotype were more frequent than in the opposite situation (14 vs 2 cases).

Conclusion: Despite the molecular classification of early-stage BC that classifies patients in well-defined prognostic subgroups, tumors are continuously changing and tumor behavior becomes more aggressive through progression. Therefore, even tumors with favorable phenotype could lose their good prognosis in locally advanced stages. Obtention of tumor tissue at metastatic sites is also mandatory to a better selection of systemic therapies in relapsed patients.

P3-05-07
Genetic Heterogeneity of Amplification Status in Breast Invasive Carcinoma with 2+ HER2 Immunostaining: What Can We Learn?
Valent A, Delaloge S, Ferech M, Bernheim A, Mathieu M-C. Institut Gustave Roussy, Villejuif, France, Metropolitan

Background: The genetic heterogeneity of HER2 gene amplification (GA) in breast cancer has previously been described, but the clinical significance of this phenomenon remains unknown. We studied the genetic categories of a series of consecutive 2+ IHC cases over 5 years, with a focus on cases with HER2 GA detected in minor clone(s). We compared the HER2 status in primary tumors and positive axillary lymph nodes (ALN) when available to test the hypothesis that HER2 amplified cells are more aggressive and metastase quicker than non amplified cells.

Material and methods: From January 1st, 2006 to May 30, 2011, 4491 invasive breast carcinomas had HER2 immunohistochemical (IHC) and/or HER2 gene status evaluation on their tumor sample.
in Institut Gustave Roussy. The distribution according to their IHC was as follows: 0 in 2915 cases (65%), 1+ in 569 cases (12.6%), 2+ in 536 cases (11.8%) and 3+ in 471 cases (10.6%). All 2+ samples were checked by Fluorescence in situ hybridization (FISH). For each case we analysed 100 invasive cells in 10 microscopic fields. Heterogeneous amplification was defined as presence of 5-50% of amplified cells (ratio>2,2) within the tumors otherwise classified as not amplified or borderline (ratio for 100 cells: <2,2 according to the ASCO 2007 and CAP criteria). The IHC 2+ tumors show five main genetic categories.

**Results**: FISH with HER2/cen17 probes can classify IHC 2+ tumors into 5 genetic categories: normal gene status (two chromosomes (chr) 17 and two HER2 genes): 90 cases; chr 17 “polyomisy”/”17q gain: 145 cases; HER2 gain: 122 cases; HER2 amplification (major clone): 135 cases and chr 17 monosomy: 44 cases. We focused our interest to IHC 2+ cases where the major clone showed no HER2 amplification, but minority clone (cut off 5%) showed the HER2 amplification (ratio >2,2 or >HER2 copies per cell). We found 48 such cases (10% of 2+ cases). These cases had been reported as not amplified (42/48) or borderline (6/48). To find out if the amplified cells are those which metastase, we checked 10 involved ALN: they all showed the similar genetic heterogeneity as a primary tumor with a global ratio <2,2. Other cases with involved ALN are in ongoing study by FISH.

**Conclusions**: The genetic heterogeneity of IHC 2+ tumors is a common event and five different genetic categories can be detected. A part of the HER2 negative cases contained one or several HER2 amplified clones. Preliminary results show a similar genetic heterogeneity of metastatic population. Our results on the small series of cases do not confirm the hypothesis that the only amplified clones metastase, but all clones (amplified and not amplified) has a metastatic capacity. A multi-center collaboration is needed to collect the high number of cases with heterogeneous amplification to:
1) Find out a proportion of such patients in HER2 IHC 2+ group (10% in our study).
2) Study a status of the gene in relapse cases.

**P3-05-08**

**Hormone Receptor Heterogeneity in Ductal Intraepithelial Neoplasia (Ductal Carcinoma In Situ) of the Breast.**

Sowden M, Flynn C, Bossuyt V, Lannin D, Chagpar AB. Yale University School of Medicine, New Haven, CT

**Background**

Ductal Intraepithelial Neoplasia [DIN] often shows heterogeneity of both morphology and nuclear grade within the same patient. It is unknown whether this implies heterogeneity with respect to hormone receptor status that may affect treatment. We sought to determine the rate of heterogeneity in terms of nuclear grade and receptor status in DIN patients.

**Methods**

A hospital tumor registry was queried for patients diagnosed with DIN between 1980 and 2010. Of the 746 patients identified, 579 (77.6%) had a concomitant invasive tumor. Of the remaining 167 patients, 70 were diagnosed prior to 2007 (when ASCO-CAP guidelines for hormone receptor measurements were released), 2 were found to have lobular intraepithelial neoplasia rather than DIN, and in 11 cases, pathology slides were not available for review. Of the 84 remaining patients with DIN, 17 (20.2%) had DIN 1 alone, 19 (22.6%) had DIN 2 alone, and 7 (8.3%) had DIN 3 alone. 41 (48.8%) of the patients had more than one grade of DIN. These patients formed the cohort of interest. Slides were reviewed by a single pathologist who evaluated ER and PR positivity within the different grades of DIN in each patient, classifying tumors staining 1% or greater as positive. Statistical analyses were performed using SPSS.

**Results**

The median patient age was 53 years old. Of the 41 patients with multiple grades of DIN, 32 (78.0%) had DIN 1 and 2, 6 (14.6%) had DIN 2 and 3, and 3 (7.3%) had DIN 1, 2, and 3 within the same tumor. 93.8%, 94.7% and 88.9% of DIN 1, 2 and 3 lesions respectively were ER-positive. A difference in hormone receptor status between different grades of DIN within the same tumor was noted in 12 (29.3%) of patients; of these 3 (10.3%) varied in ER status and 10 (34.5%) varied in PR status. Of the 2 patients with ER-negative DIN 1, both had ER-positive higher grade DIN within the same tumor. Of the 2 patients with ER-positive DIN 2, one had ER-positive DIN 1 while the other had ER-negative DIN 3. Therefore, of the total 41 patients, 40 (97.6%) had at least one component of DIN that was ER-positive, and evaluating more than one grade of DIN for ER allowed 75% of patients with at least one ER-negative component to be offered hormonal therapy.

**Conclusions**

Nearly 50% of patients with DIN will have more than one nuclear grade in the same tumor. These different nuclear grades vary in hormone receptor status in approximately 30% of patients. The majority of patients (97.6%) have at least one component of their DIN that is ER-positive. For those who have at least one component that is ER-negative, up to 75% may be offered hormonal therapy by considering other grades of DIN in treatment decision-making.

**P3-05-09**

**Comparison of Clinical Features and Patterns of Recurrence in Triple Negative Breast Cancers in Relation to Other Breast Cancers.**

Tosello de Oliveira C, Barbosa EM, Costa de Andrade J, Lyra EC, Felzener MC, Krutman Zweibil D, Grosso SHG, Sampaio Goes de Oliveira R, Sampaio Góes JC. Instituto Brasileiro de Controle do Cancer, Sao Paulo, Brazil

**Background**: Breast cancer is an heterogeneous disease that have several and different biological characteristics and clinical behaviours. Of these, triple negative breast cancer represents 10-17%.This kind of tumor is characterized by estrogen, progesterone and HER2 receptor negativity and they are very aggressive, associated with poor prognosis, younger patients, high incidence of metastases and shorter relapse free survival. Triple negative breast tumours are more likely to experience local recurrence and distant relapse than other breast tumours, and require a more aggressive intervention.

**PURPOSE**: To compare the incidence of metastases, clinical features and outcome among patients with triple negative breast cancer and women with other types of breast cancer.

**METHODS**: This is a retrospective cohort analysis from 3893 patients with invasive breast cancer, diagnosed between January 2000 and April 2011 and attended at Brazilian Institute of Cancer Control (IBCC) in Sao Paulo, Brazil. Five hundred of these tumors were triple negative. We made a correlation of clinical variables such as age, tumor stage (TNM) and analysis of last follow-ups, such as metastases, disease free survival, death for tumor, death for other reasons, lost of follow-up, hygiene and diet regime (RHD) and local recurrence.

**Results**: The median follow-up for the cohort was 4.29 years. The mean age at diagnosis in triple negative group was 54.8 and for the others group was 57.1 years. Subgroup analysis of tumor grade showed that the triple negative breast tumors were diagnosed...
later (Stage II and III; p<0.0001). There was no difference in the appearance of the two types of tumors, when considering diagnose age. The incidence of metastases, death for tumor and free survival in triple negative tumors, respectively; 25.6%, 13.8% and 57.2% (p<0.0001) demonstrates the aggressiveness of this kind of tumor.

<table>
<thead>
<tr>
<th>Characteristics of triple negative versus other breast cancers</th>
<th>Other</th>
<th>Triple Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>3393</td>
<td>N 500</td>
</tr>
<tr>
<td>N %</td>
<td>N %</td>
<td>p (Chi-square)</td>
</tr>
<tr>
<td>Average Diagnose Age</td>
<td>1071</td>
<td>54.81</td>
</tr>
<tr>
<td>Average Follow-up (Years)</td>
<td>8.29</td>
<td>8.28</td>
</tr>
<tr>
<td>Tumor Grade</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>665</td>
<td>19.65%</td>
</tr>
<tr>
<td>II</td>
<td>1486</td>
<td>45.40%</td>
</tr>
<tr>
<td>III</td>
<td>1067</td>
<td>31.45%</td>
</tr>
<tr>
<td>IV</td>
<td>173</td>
<td>5.10%</td>
</tr>
<tr>
<td>Disease Grade</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metastases</td>
<td>843</td>
<td>20.10%</td>
</tr>
<tr>
<td>Disease free survival</td>
<td>2225</td>
<td>65.05%</td>
</tr>
<tr>
<td>Death for Tumor</td>
<td>591</td>
<td>17.69%</td>
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<tr>
<td>Death for other reason</td>
<td>157</td>
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<td></td>
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<tr>
<td>RHD</td>
<td>5</td>
<td>0.15%</td>
</tr>
<tr>
<td>Local recurrence</td>
<td>0</td>
<td>0.03%</td>
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</tbody>
</table>

Results of last Follow-up:

<table>
<thead>
<tr>
<th>Follow-up</th>
<th>N</th>
<th>%</th>
</tr>
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<tbody>
<tr>
<td>No Follow-up</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>RHD</td>
<td>5</td>
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<tr>
<td>Local recurrence</td>
<td>0</td>
<td>0.03%</td>
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P3-05-10
Standardized Quantitative Methods for Investigating the Intratumor Heterogeneity of HER2 in FFPE Breast Cancer Specimens Utilizing the Vectra System, inForm and AQUA Technology.

Hoyt CC, Gustavsson MD, Davis WL, Lane KA, Scott CG, Graves, Jr LL, Caliper Life Sciences, Hopkinton, MA; HistoRx, Branford, CT

Background: While intratumoral heterogeneity of HER2 gene expression is well documented, the HER2 intratumor protein expression heterogeneity and its clinical relevance is largely unknown but generally believed to be worth of investigation to ascertain its impact on HER2 targeted treatment outcome. Methods for investigating intratumor protein expression heterogeneity must preserve tissue architecture yet provide quantitative protein measurement. Immunohistochemistry (IHC) generally lacks standardization and quantitative capabilities and furthermore, current scoring methods do not account for, or report, heterogeneity. Robust and automated methods for characterizing and quantification of heterogeneity are needed. Material and Methods: Whole tissue samples stained for HER2 protein expression by fluorescent quantitative IHC methods were analyzed by digital imaging microscopy (VeCTra system with inForm software, Caliper, Hopkinton MA) providing automated pattern-recognition-based selection of area of interest and field of view sampling strategies. Quantitative image analysis (AQUA technology, HistoRx, Branford, CT) for quantitative measurement of HER2 protein expression as well as objective, quantitative determination of heterogeneity were performed. Requirements for percent tumor sampling at high-power to capture heterogeneity were assessed, to support optimization of slide scanning. Results: Heatmap representation of HER2 expression levels, and a statistical heterogeneity score developed based on the Simpson’s biodiversity index (Simpson 1949) demonstrate visually as well as quantitatively HER2 heterogeneity in breast cancer tissues. Heterogeneity as a function of % area needed for accurate determination of whole section score along with confidence intervals as a function of percentage tumor analyzed inform appropriate sampling strategies. Conclusion: This study demonstrates the suitability of standardized quantitative methods for measuring heterogeneity and the feasibility of its use in investigations exploring the clinical relevance of HER2 intratumor heterogeneity with respect to both measurement strategies as well as treatment outcome. Through characterization of heterogeneous HER2 expression in tumors and optimized measurement strategies, it should be possible to design optimized treatment strategy aimed at treating the whole tumor as a function of objective quantitative assessment of HER2 expression.

P3-06-01
Next Generation RNA Sequencing Reveals Changes in Gene Expression and Alternative Splicing upon Brief Exposure to Therapy in Early Breast Cancer.

Varadan V, Kamalakaran S, Janevski A, Banerjee N, Lezon-Geyda K, Bossuty V, Flowers D, Sikov W, Abu-Khalaf M, Rizack T, Dimitrova N, Harris LN. Philips Research North America, Briarcliff Manor, NY; Yale University School of Medicine, New Haven, CT; Brown University School of Medicine, Providence, RI

Background:
The use of next generation RNA sequencing (RNA-seq) allows for the characterization of the transcriptome at levels of detail unachievable by array-based technologies. RNA-seq analysis can quantify expression of novel transcripts and alternatively spliced isoforms in addition to known genes. Alternative splicing allows for flexibility in production of protein isoforms and is frequently dysregulated in cancer. As splice variants may play a role in response to therapy (Solier, et al, Cancer Res., 2010), we studied differential gene and isoform expression in breast cancers after one dose of treatment, prior to a course of preoperative therapy.

Methods:
We sequenced transcriptomes of core biopsy samples from 8 breast cancer patients enrolled in a preoperative clinical trial using trastuzumab (HER2 positive) or bevacizumab (HER2 negative) with chemotherapy. Tumor core biopsies were taken before and after 10 days of either biologic or nab-paclitaxel treatment and stored in OCT compound. Total RNA was extracted, amplified and libraries were constructed for the 16 samples using TrueSeq (Illumina). Paired-end sequencing was performed on the Illumina GAII platform with read length of 74bp. Sequence data was mapped using TopHat and transcript abundance in FPKM units (Fragments per kilo-base of mRNA per million reads) estimated for a total of 22,160 unique genes and 34,449 unique transcripts from RefSeq. Differential expression of transcripts between baseline and 10-day samples was estimated using t-statistics with read-counts modeled as a Poisson distribution. Differentially expressed transcripts were selected at a significance level of 0.05 after multiple testing correction.

Results:
Each sample had on average 46 million paired-end reads, of which on average 70% were mappable to the human reference genome (UCSC, hg19). A median of 138 (range 68-948) transcripts were analyzed for differential expression upon brief exposure to chemotherapy. GO analysis showed enrichment of cell adhesion, apoptosis, differentiation and cell proliferation pathways. Interestingly, the isoforms of several known cancer genes such as TP53 were seen in all treatment types. Certain isoforms were only seen to change upon brief exposure to chemotherapy such as BCL2 whereas TNF ligand and PCDH isoforms showed significant change only with biologic agents.
Conclusions:
These results suggest that recurrent changes in both canonical genes and splice variants occur over the course of treatment in early breast cancer. This underscores the value of RNA-seq to provide novel information that may be clinically useful. Brief exposure to monotherapy prior to combination treatment may provide important mechanistic insights and produce predictive biomarkers. Biologic treatments may produce unique changes that can only be discovered with novel next generation sequencing techniques.

P3-06-02
Identification of Redundant, Tumor Subtype Specific Fusion Transcripts in Primary Breast Tumors.

Background: The role of fusion genes and associated fusion transcripts has long been recognized in hematopoietic malignancies. Until quite recently it has been difficult to detect such events on a genomic scale in solid tumors. Consequently, little is known about the potential role of fusion genes, transcripts, and proteins as driver mutations, biomarkers, or therapeutic targets in breast cancer.

Methods: We have developed a novel analytical pipeline, Snowshoes-FTD, for detection of fusion transcripts in breast cancer cell lines and tumor samples (Asmann, et al. NAR 2011; May 27 ePub ahead of print). Preliminary analyses have been carried out with a panel of 8 each ER+, HER2+, and triple negative (TN) primary breast tumors, 8 primary human mammary epithelial cell (HMEC) lines from biopsy samples, plus 16 normal tissues from the Illumina Body Map dataset.

Results: We have identified 120 redundant, tumor-specific fusion transcripts, expressed in two or more tumors and in no non-transformed samples. Sixteen of these represent intrachromosomal fusions and 104 arise from fusion of transcripts that map to two different chromosomes. Every breast tumor expressed one or more fusion transcripts. Twenty-nine fusion transcripts appeared to be tumor subtype specific. Among these, we have identified 2 HER2+, 10 ER+, and 17 triple negative specific redundant transcripts. In general, HER2+ tumors expressed fewer fusion transcripts (range 4 to 28/tumor) compared to TN (range 11 to 44/tumor). Chromosomal distribution patterns were also markedly different among the tumor subtypes. For example, ER+ tumors expressed a preponderance of redundant fusion transcripts that involve chr1 and 2, whereas TN tumors had no fusion transcripts that map to either chromosome. Conversely, the predominant locus for TN fusion transcripts was chr19, which contains only one HER2+ fusion and no ER+ fusion transcripts.

Conclusions: Primary breast tumors express many chimaeric transcripts, which we presume to arise primarily from genomic rearrangements. The majority of these transcripts are redundant, and a subset are tumor subtype specific. These transcripts may mark regions of chromosomal instability. HER2+ tumors, in general, appear to evidence less chromosomal instability, as inferred from fusion transcripts; although some HER2+ tumors appear to be quite unstable. TN tumors contain many more redundant fusion transcripts, implying increased genomic instability, particularly in chr19. We conclude that these fusion transcripts represent a class of heretofore unrecognized biomarkers that may be used for sub-classification of breast tumors. Some of these transcripts appear to encode proteins that may function as tumor-subtype-specific driver mutations and may have potential as therapeutic targets in breast cancer.

P3-06-03
Hypodiploidy, 1pter Loss and Inactive X Chromosome Retention Are Associated with BRCA1 Somatic or Germline Inactivation in Basal-Like Breast Carcinomas: Proposal for a New BRCA1ness Genomic Signature.
Manié E, Popova T, Vincent-Salomon A, Dubois T, Delattre O, Sastre-Garau X, Stoppa-Lyonnet D, Stern M-H. Institut Curie, Paris, France; INSERM, Paris, France; Université Paris-Descartes, Paris, France

Basal-Like Carcinomas (BLCs) are high grade ductal carcinomas identified by large scale transcriptomic analyses among carcinomas with triple-negative phenotype (ER- PR- HER2-). A particular relationship between this phenotype and the BRCA1 breast cancer susceptibility gene has been described based on several observations including high genomic similarities of sporadic and BRCA1 BLCs. BRCA1 mutated patients have been shown to strongly benefit from treatments using PARP inhibitors. However, significant benefit of this treatment was not demonstrated in sporadic triple-negative breast cancers in association to standard chemotherapy in phase III clinical trials, which emphasizes the necessity for a better selection of DNA repair deficient tumors. The goal of this study was to determine whether BRCA1 inactivation leads to particular genomic alterations that could be used to identify BRCA1 deficient sporadic BLCs.

Genomic profiling was performed using SNP-arrays (Affymetrix and Illumina) and high quality profiles were obtained for 60 BLCs with more than 35% of tumor cells. The series contained 28 BRCA1 BLCs and 32 sporadic BLCs consisting in 10 sporadic BLCs with BRCA1 somatic inactivation by promoter methylation (BRCA1-like BLCs) and 22 sporadic tumors without BRCA1 methylation (nonBRCA1 BLCs). Genomic data was mined with GAP methodology (Popova et al, Genome Biol 2009), which allows absolute copy-number evaluation with DNA content calculation and clarification of Loss Of Heterozygosity (LOH) status.

Genomic patterns of BLCs were characterized by frequent and highly recurrent allelic losses. Comparison between BRCA1, BRCA1-like and nonBRCA1 BLCs confirmed their overall similarity, and identified few significant differences enhanced when BRCA1 and BRCA1-like BLCs were considered as one group. Firstly, hypodiploidy characterized BLCs with BRCA1 germline and/or somatic inactivation. Secondly, the 1p region was found more often lost in BRCA1 and BRCA1-like BLCs than in nonBRCA1 BLCs. Thirdly, retention of inactive X chromosome was a characteristic of BLCs with BRCA1 germline and/or somatic inactivation. Finally, a BRCA1ness score based on the above described difference is proposed.

In conclusion, despite a strong similarity of genomic patterns in BLCs, BRCA1 inactivation leads to few key genomic aberrations which constitute a new BRCA1 molecular signature namely hypodiploidy, 1p loss and inactive X chromosome retention. This signature needs to be further evaluated in terms of response to PARP inhibitors in order to evaluate its capacity to identify patients that will benefit from this treatment.

P3-06-04
Sno/miRNA Expression Via Next Generation Sequencing: Variation in Patients before and after Treatment.
Banerjee N, Kamalakaran S, Vayadan V, Janevski A, Lezon-Geyda K, Bossuyt V, Flowers D, Sikov W, Abu-Khalaf M, Rizack T, Harris L, Dimitrova N. Philips Research North America, Briarcliff Manor, NY; Yale University School of Medicine, New Haven, CT; Brown University School of Medicine, Providence, RI

Background: Aberrant expression of small RNA molecules has been shown in breast cancer. It is yet unclear if variation exists in...
small RNA molecule expression in response to treatment. Since next generation sequencing offers more globally sensitive detection of sno and miRNAs, we performed an RNA-seq study to explore patients pre- and post-brief exposure to treatment.

Methods: We sequenced transcriptomes of frozen biopsy samples from 8 breast cancer patients enrolled in a clinical trial for neoadjuvant therapy using trastuzumab (HER2 positive) or bevacizumab (HER2 negative) with chemotherapy. Tumor core biopsies were taken before and after 10 days of either biologic or nab-paclitaxel treatment and stored in OCT compound. Total RNA was extracted and libraries were constructed for the 16 samples using TruSeq (Illumina). We performed 74bp paired-end sequencing with the Illumina GAI1 platform. Sequences were aligned to the sno/miRNA track (containing 928 miRNAs and 413 snoRNAs) in UCSC and read counts were determined using Bowtie. We performed differential miRNA and snoRNA expression analysis pair-wise in all pre- and post-therapy samples. Given that miRNA deregulation relies on their protein-coding gene targets, we analyzed the predicted targets of the significantly varying miRNAs for functional enrichment.

Results: Each sample had on average 46 million paired-end reads, of which on average 70% were mapped to the human genome. Overall, we detected 138 miRNAs in at least one sample, with each sample expressing 33 miRNAs on average. We detected a total of 11 miRNAs (7%) that showed significant differential expression with treatment. Interestingly, 6 of these miRNAs varied in all patients. The predicted targets of these miRNAs were enriched in DNA-dependent transcription, gene expression, cell proliferation and cell communication. Similarly, we detected 202 snoRNAs in at least one sample, with each sample expressing 87 snoRNAs on average. Of these, we found 21 snoRNAs (10%) to vary significantly upon treatment and 6 of these snoRNAs showed expression changes in all patients.

Conclusions: These results suggest that variation in sno/miRNA expression may play a role in response to treatment. The consistent variation of sno/miRNAs in response to treatment suggests shared small RNA-mediated mechanisms. If validated, these results suggest that next generation sequencing technologies will allow lead to improved methods of stratifying, subclassifying and managing breast cancer.

P3-06-05
Comparison of Oncotype DX® Recurrence Scores between Surgical and Core Biopsy Specimens in Breast Cancer Patients.
Stull TS, Goodwin MC, Anderson JM, Baehner FL, Sing AP, Yoshizawa CN, Barrio AV, Frazier TG. The Bryn Mawr Hospital, Bryn Mawr, PA; Genomic Health, Inc, Redwood City, CA

Introduction: The Oncotype DX® 21 gene assay is usually performed on surgical resection specimens (SRx) for patients (pts) with early stage, estrogen receptor positive (ER+) breast cancer to predict the risk of recurrence and likelihood of benefit from adjuvant chemotherapy (CT) and hormonal therapy (HT). Using the Recurrence Score (RS) to aid in making a decision to administer preoperative CT requires assaying the core biopsy specimen (CBx). The objective of this study is to compare the RS obtained from paired CBx and SRx specimens. Methods: 25 pts with invasive breast cancer diagnosed by CBx with a subsequent Oncotype DX® 21 gene assay performed on the SRx were identified. Fixed, paraffin embedded tissue from these paired CBx were sent to Genomic Health. The H&E slide from the paired SRx sample sent for the RS was used to compare the histology with the paired CBx. The RS from the CBx and SRx pairs were examined descriptively with scatterplots and Pearson correlation coefficients.

Results: Median age of diagnosis was 64 (range 39-78). Sufficient RNA was obtained from all but 1 of the CBx. H&E from CBx and SRx were histologically dissimilar for 3 of 24 pairs with RT-PCR data. The Pearson correlation for RS between CBx and SRx was 0.83 (95% CI 0.64, 0.92) for all 24 pairs. The distribution of RS groups from CBX was 19 low (RS<18), 3 intermediate (RS 18-30), 2 high (RS≥31). The RS group from SRx was unchanged for 22 (92%) pts. Two pts switched RS groups, CBx RS 5 to SRx RS 18 and CBx RS 8 to SRx RS 24; only the latter is clinically meaningful because the former straddles the low-intermediate cutoff. Correlations between CBx and SRx for individual single gene scores were 0.84 (95% CI 0.65, 0.93) for ER, 0.83 (95% CI 0.64, 0.92) for PR, and 0.82 (95% CI 0.61, 0.92) for HER2. Similar results were obtained excluding the 3 histologically dissimilar pairs.

Conclusion: The RS’s obtained from CBx’s were consistently similar to the RS’s obtained from the paired SRx. Using an RS obtained from a CBx in pts with ER+ disease to make a decision regarding neoadjuvant CT or HT is clinically acceptable, thus, giving physicians an additional tool to improve pt care and outcomes.

P3-06-06
Comparison of Gene Expression Profiles of Lymph Node Positive and Lymph Node Negative ER Positive Breast Tumors in Pre- and Postmenopausal Women.
Rapuri PB, Xing L, Brilhart G, Deyarmin B, Kvecher L, Hu H, Hooke JA, Shriver CD, Mural RJ. Windber Research Institute, Windber, PA; Walter Reed Medical Center, Washington, DC

Background: Breast cancer is the most common female cancer in US and is the second leading cause of cancer related death in women. Metastases are the primary cause of cancer morbidity and mortality. Axillary lymph node (LN) status has long been used as a prognostic factor for breast cancer. The molecular mechanisms that control LN metastasis remains poorly understood. To better understand the various genes and regulatory pathways that drive breast cancer LN metastasis, we compared the gene expression profiles between breast tumors that have metastasized to the LNs and those which have not in pre- and postmenopausal women.

Material and Methods: Tumor cells were isolated from the primary tumors (ER+) of postmenopausal node positive (PMNN; N=20), postmenopausal node negative (PMNN; N=19), premenopausal node positive (PRPN; N=18) and premenopausal node negative (PRPN; N=16) women using laser capture microdissection. RNA isolation was performed using the RNAqueous®-micro kit (Ambion, Austin, TX). Total RNA was converted to Biotin-labelled aRNA using two rounds of amplification with MessageAmp II aRNA amplification kit (Applied Biosystems, Foster City, CA). The aRNA concentration was determined by Nanodrop 1000 and the quality was assessed with a Bioanalyzer. The aRNA was fragmented and hybridized to Human Genome U133 Plus 2.0 GeneChip (Affymetrix, Santa Clara, CA). Microarray raw data were analyzed using a variety of R programming packages for probe density processing, background correction, normalization, quality control/quality assessment, and calculation of gene expression value, etc. To identify differentially expressed genes, Wilcoxon rank sum test with FDR (false discovery rate) control was performed for pair-wise comparison between different groups. Functional analyses were performed on the identified statistically significant differentially expressed genes to search for the functional categories and pathways in which they are involved and further understand their potential roles in breast cancer metastatic process. Results: Multivariate data mining (hierarchical clustering analysis and principal component analysis, etc) revealed that in postmenopausal...
women, the node positive and node negative women are well separated while this was not the case in premenopausal women. Further analysis of the PMNN and PMNP groups to identify differentially expressed genes (with at least a 1.5 fold difference) at FDR =0.1 showed that 232 genes were upregulated and 470 genes were downregulated in PMNP vs PMNN groups. Gene function analysis revealed that genes down regulated in the PMNP group compared to PMNN are related to extracellular matrix, cell adhesion, EGF-like pathway, cytoketoson etc, while the over-expressed genes are related to cell cycle and cell division, chromosome condensation, etc.

**Discussion:** The ability to differentiate lymph node positive cases from lymph node negative cases in ER+ breast cancer based on transcriptional profiling may have an impact on the clinical management of ER+ breast cancer cases. Having transcriptional profiles that identify ER+ tumors likely to have poor outcomes would suggest more aggressive treatment for such patients.

**P3-06-07**

Integrated Genomic and Pathway Analysis Reveals Key Pathways across Breast Subtypes.

Benz S, Sanborn JZ, Vaske C. Five3 Genomics, LLC, Santa Cruz, CA

Cancer is a disease of genomic perturbations that lead to dysregulation of multiple pathways within the cellular system. While common pathways are believed to be shared within specific cancer types, the mechanisms behind why particular patients respond differently to treatment is not well understood. Genomics studies such as The Cancer Genome Atlas (TCGA) and Stand Up To Cancer (SU2C) attempt to address this issue by collecting large-scale whole-genome measurements of mRNA expression, DNA copy number, and epigenetic features. Common analysis of these measurements integrates data across multiple samples to distinguish signal from noise. However, serious challenges remain in identifying genomic features and pathways significant for prognosis and clinical treatment classifications.

We have created the Five3 Analysis Pipeline to streamline discovery of individual samples’ mutations, small indels, copy number alterations, genome rearrangements, expression changes, and resulting pathway activities. This pipeline is capable of processing and integrating data from both next generation sequencing and microarray platforms in the analysis of single or multiple tumor samples. Our sequence analysis corrects for both tumor sample impurity and germline variation to accurately identify somatic mutations present in the tumor. Our pathway analysis incorporates gene copy number, mutations, expression, and promoter methylation on a superimposed pathway constructed from several curated pathway databases in a sample-specific manner.

By applying this pipeline to the TCGA breast cancer datasets, we recapitulate established breast subtypes at a pathway-dependent level. For example, basal tumors appear enriched for proliferation pathways compared to luminal samples within this cohort. Expanding the pathway analysis to include TCGA lung cancer samples, we find similar subnetworks activated between basal and squamous lung and between luminal and lung adenocarcinomas. This hints at similar genomic mechanisms for these subtypes independent of tissue of origin. Finally, by analyzing genomic alterations across all breast cancers we see mutational clusters in PIK3CA that correspond with publicly-available hotspots [1]. As suggested by previous reports [2], we find that samples with mutations clustered in exon 10 exhibit differential pathway activities relative to those samples with mutations clustered in exon 21, independent of subtype and TP53 mutation status. These results show the power of this integrated genomic platform in elucidating pathway signatures and the need to consider cross cancer analyses to identify shared tumorigenic mechanisms that may suggest common therapeutic targets.


**P3-06-08**


The South Sweden Cancerome Analysis Network - Breast (SCAN-B) Initiative is a multidisciplinary network of clinical providers of breast cancer (BC) treatment and pre-clinical scientists whose multiyear purpose is to 1) prospectively collect and analyze the “omes” of a very large, consecutive, and population-based sample of BCs for translational research; 2) utilize this genomic data to develop new clinically-relevant biomarker assays; and 3) to build the infrastructure for future real-time clinical implementation of resultant biomarkers for individualized treatment. Patient enrollment began in the Fall of 2010 at the seven BC surgical units of the South Sweden Healthcare Region, where approximately 1500 new breast cancer diagnoses are treated yearly following the guidelines of the South Sweden Breast Cancer Group. Currently, tumor and blood samples are being collected at the rate of 80-100 patients per month, which represents approximately 75% of the catchment population. SCAN-B will initially focus on tumor transcriptome analysis using mRNA-sequencing on Illumina HiSeq 2000 instruments, and in the future multimodal data generated from other genomic platforms will also be integrated. Here we present initial experiences from this multidisciplinary collaboration including descriptions of the clinical routines, specimen handling, laboratory processing, mRNA-seq data quality control, short- and long-term projects and future directions. We believe large initiatives like SCAN-B could significantly reduce the time to discovery, validation, and clinical implementation of more powerful diagnostic, prognostic, and treatment-predictive tests for breast cancer.

**P3-07-01**

Are the Findings of ASOCOG Z0011 Applicable to District General Hospital Breast Unit – And How Should They Change Our Practice? Olsen SB, Amr B, Omar A, Smith J, Thomson S, Monib S, Lai LM. West Hertfordshire NHS Trust, St Albans, Hertfordshire, United Kingdom

Background: There has been considerable debate on the management of the axilla in breast cancer patients with lymph node metastasis found on sentinel lymph node biopsy (SLNB) following publication of the ACOSOG Z0011 study. This study conclude that axillary lymph node dissection (ALND) does not add any benefit to overall and disease free survival in some patients with positive SLNB. However it is not know if the patient characteristics of this study are transferable to other settings. A previous UK study has suggested that only a minority of patients from an academic specialist centre fit the ASOCOG Z0011 criteria and that the patient populations may not be
comparable. The aim of our study was to assess the applicability of the Z0011 study to our patient population of a large District General Hospital in the UK and to what extent it should influence our practice. Methods: The Z0011 eligibility criteria for inclusion in analysis were applied to all patients undergoing SLNB for invasive cancer at West Hertfordshire Breast Unit from 2007-2011. These were: no neo-adjuvant chemotherapy, clinical T1 and T2 tumours, breast conserving surgery followed by whole breast radiotherapy and 1-3 positive nodes on SLNB. Patent characteristics and results were compared using fisher’s exact test.

Results: In our unit a total of 434 patients underwent SNLB of whom 64(14.7%) met the inclusion criteria of Z0011. Our patient population was comparable to that of Z0011 with regards to lymphovascular invasion, proportion of patients with micro-metastases and those with additional lymph nodes found on completion ALND. Our patients had significantly more T2 tumours. The only other statistically significant difference was in the proportion of estrogen receptor (ER) and progesterone receptor (PR) positivity.

<table>
<thead>
<tr>
<th>Tumour characteristics</th>
<th>Z0011</th>
<th>Our Patients</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>70.6% (305/436)</td>
<td>83.5% (80/96)</td>
<td>P 0.0002</td>
</tr>
<tr>
<td>T2</td>
<td>29.4% (126/436)</td>
<td>16.5% (15/96)</td>
<td>P 0.0002</td>
</tr>
<tr>
<td>ER+/PR+</td>
<td>61.9% (270/436)</td>
<td>75.0% (72/96)</td>
<td>P 0.002</td>
</tr>
<tr>
<td>ER+/PR-</td>
<td>14.7% (64/436)</td>
<td>4.7% (4/96)</td>
<td>P 0.03</td>
</tr>
<tr>
<td>LVI present</td>
<td>25.9% (113/436)</td>
<td>39.1% (35/90)</td>
<td>P 0.38</td>
</tr>
<tr>
<td>LVI absent</td>
<td>47.7% (208/436)</td>
<td>54.7% (51/95)</td>
<td>P 0.38</td>
</tr>
<tr>
<td>Micro-metastases</td>
<td>44.8% (164/366)</td>
<td>37.5% (35/94)</td>
<td>P 0.41</td>
</tr>
<tr>
<td>Patients with additional nodes found on ALND</td>
<td>27.3% (97/355)</td>
<td>25.8% (16/64)</td>
<td>P 0.65</td>
</tr>
</tbody>
</table>

Conclusion: Only a small proportion (14%) of patients undergoing SNLB in our patient population fit the criteria for Z0011. This was a higher proportion than in the previous UK study of this type. Similar to this study we found the proportion of patients with T2 tumours were greater in our patient population compared to Z0011. This is most likely a reflection of this unit not being a screening centre and brings into question the international generalisability of Z0011. However the only other significant difference in our patients was a lower risk profile in terms of hormone receptor positivity with more ER/PR positive and fewer ER/PR negative patients. The implications of Z0011, will in our practice, not make a significant difference to the rate of ALND. We are however considering changing our patient management based on the result of Z0011 and this current study of our population, to offer no further axillary surgery to patients over 50 with T1 and ER/PR positive tumours who otherwise fit the Z0011 protocol.

P3-07-02
Prediction of Non-Sentinel Lymph Node Status in Breast Cancer Patients with a Micrometastatic Sentinel Lymph Node Determined by the One Step Nucleic Acid Amplification (OSNA) Assay.
Di Filippo F, Casini B, Gallo E, Terrenato I, Botti C, Motolese M, Pescarmona E, Maranondo F, Bugliosi S. Regina Elena National Cancer Institute, Rome, Italy; Regina Elena National Cancer Institute, Italy

Background: Axillary lymph node dissection (ALND) may not be necessary in women with breast cancer (BC) who have micrometastasis in a sentinel lymph node (SLN), owing to the low risk of non-SLN (NSLN) involvement. In our Institute we validated and adopted the new molecular diagnostic tool OSNA (One Step Nucleic Acid Amplification) based on the quantitative measurement of Cytokeratin 19 (CK19) mRNA. The aims of our work in a subgroup of women with micrometastatic SLN, were: 1) to correlate the copy numbers of CK19 mRNA with the risk of additional positive NSLNs; 2) to assess the relationships between the molecular subtype classification based on the immunohistochemistry phenotypic patterns and the probability of a positive ALND; 3) to verify whether a combination of the new above mentioned parameters is able to identify a subgroup of patients with a micrometastatic SLN and a negligible risk of positive NSLNs in whom ALND may be avoided.

Material and Methods: The intraoperative clinical study was conducted on 901 fresh SLNs from 709 consecutive patients with clinically node negative BC. The SLN lysates were analyzed by OSNA assay. If the CK19 mRNA copy number/mL lysate was less than 250 copies/mL, the result was regarded as negative (-); copy numbers between 250 and 5000/mL were regarded as micrometastasis (+), and copy numbers greater than 5000/mL as macrometastasis (++). We analyzed only patients with a micrometastatic SLN. Complementary ALND was performed concurrently in case of OSNA assay positivity and the probability of having a positive lymph node axillary dissection was calculated by the unconditional logistic regression model. This series of BC patients were divided into four main subtypes taking in account the BC classification as defined by a combination of estrogen, progesteron receptors and HER2 status evaluated by immunohistochemistry (IHC) and confirmed by FISH in case of IHC-HER2 2+

Results: OSNA positivity for micrometastasis was reported in 91/709 cases (12.8%). The number of patients with positive ALND was 20 (22%). The statistical analyses showed that the metastatic involvement of NSLNs is associated with SLNs with a high copy numbers (>2000) of CK19 mRNA together with luminal B subtype. Otherwise none of the luminal A patients with a positive SLN but presenting a copy number <1000, had a positive NSLNs.

Conclusions: We showed that biologically-driven analyses may be able to build new models with higher performance in terms of breast cancer axillary status prediction after positive SLN biopsy for micrometastasis. The copy numbers of CK19 mRNA and the molecular subtypes are more advantageous than traditional parameters because they are not pathologist-dependent and therefore they are more reliable and reproducible.

P3-07-03
One-Step Nucleic Acid Amplification (OSNA) for the Diagnosis of Sentinel Lymph Nodes of Breast Cancer – Results of the China Multicenter Study CBCSG-001c
Wang YS, Ouyang T, Wu J, Liu YH, Cao XC. Shandong Cancer Hospital & Institute, Jinan, Shandong, China; Beijing University Cancer Hospital, Beijing, China; Fudan University Cancer Hospital, Shanghai, China; Guangdong General Hospital, Guangzhou, Guangdong, China; Tianjin Midical University Cancer Hospital, Tianjin, China

Background: With the adoption of sentinel lymph node (SLN) biopsy as the standard of care, there is an increasing need for the rapid and accurate intra-operative diagnosis of SLNs. CBCSG-001c was a prospective multicenter trial to validate the One-step nucleic acid amplification (OSNA) assay in China. The primary endpoint was the concordance rates of intraoperative OSNA assay with the in-depth permanent histological analyses based both on cases and SLNs.

Methods: From Feb. to Dec. 2010, 1188 SLNs from 552 breast cancer patients were enrolled in the CBCSG-001c study at 5 centers. SLNs were cut into alternating ~2mm sections. One half of the sections were sampled for H&E, with 4 sections at different intervals. The other half was fully tested with the OSNA assay. Predetermined cutoffs were calibrated so only metastases >0.2 mm were detected.

Results: The concordance rate was 89.1% (95% CI, 86.3-91.5%), sensitivity 87.7% (95% CI, 81.0-92.7%), and specificity 89.6% (95% CI, 86.3-92.4%) based on 552 cases, and the concordance...
rate was 91.4% (95% CI, 89.7-92.9%), sensitivity 83.7% (95% CI, 77.7-88.6%), and specificity 92.9% (95% CI, 91.1-94.4%) based on the 1188 SLNs. This quantitative molecular assessment allows the distinction of the size of the metastasis, and the PPV of OSNA [-+] for macrometastases was 83.2% (95% CI, 75.0- 89.1%). Discordant results were thought to be partly due to the fact that different tissue sections were used for OSNA assay and histology, and SLNs with ITCs were not considered as histological positive nodes. After discordant case investigation, the sensitivity of OSNA assay was significantly higher than that of intraoperative frozen section and touch imprint cytology.

Discussion: As the largest OSNA study to date, our results, together with that of Japan, Germany, and France study, proved the OSNA assay based on CK19 mRNA expression to be a reliable and standardized tool for the intraoperative detection of SLN metastases of breast cancer patients as compared to in-depth permanent histology. The high sensitivity of OSNA assay means reducing the risk of second operation for ALND, medical care costs and patients anxiety.

P3-07-04
Does Omission of Axillary Dissection after a Positive Sentinel Node Biopsy Influence Indication to Adjuvant Chemotherapy in Operable Breast Cancer Patients?

Introduction: Based on the recently published ACSOG Z0011 study (JAMA 2011;305:569), axillary dissection (AD) may be avoided in breast cancer (BC) patients with a clinically negative axilla and a positive sentinel lymph node (SLN) biopsy receiving breast conserving surgery (BCS). Because the number of positive axillary lymph nodes (ALN) is a widely accepted prognostic marker, we evaluated the potential impact of omission of AD on indication to adjuvant chemotherapy (ACT).

Patients and methods: Among 1497 patients operated at our Institution over 10 years, we identified 321 patients fulfilling the inclusion criteria of the ACSOG Z0011 study (BCS plus SLNB, cT1-2, cN0 breast cancer and 1 to 2 positive SLN). All patients underwent AD. Each case, which was anonymized, was reviewed by our breast team in two rounds. In the first round, patient age, histopathology, linfovascular invasion, tumor grade, hormone receptor, HER2, and ki67 status and number of positive SN nodes (micro and/or macrometastatic) were available. In the second round, the information on ALN was added. At each round, the panel chose between three indications: 1) Recommend ACT; 2) Discuss ACT; 3) No ACT.

Results: SN was micrometastatic in 145 (45%) and macrometastatic in 176 patients (55%). ALD revealed non-SNs metastases in 96 patients (30%). Forty-four of these patients had >3 positive ALN (range 4-24).

Indications at round 1 and 2 are summarized below.

<table>
<thead>
<tr>
<th></th>
<th>Round 1</th>
<th>Round 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Recommend ACT</td>
<td>Discuss ACT</td>
</tr>
<tr>
<td>Totals round 1</td>
<td>357s</td>
<td>26 (41)</td>
</tr>
<tr>
<td>Round 1</td>
<td>Discuss ACT</td>
<td>26 (41)</td>
</tr>
<tr>
<td>No ACT</td>
<td>9 (11)</td>
<td>9 (11)</td>
</tr>
<tr>
<td>Totals round 2</td>
<td>177</td>
<td>40</td>
</tr>
</tbody>
</table>

As a result of the disclosure of the total number of involved ALN, a change in the indication occurred in 51 patients (16%). The most frequent change was a recommendation to ACT (35 patients, 69% of the changes). Among these patients, 9 changed from No-ACT to recommend ACT. All except one change in the indication to ACT occurred in patients with immunohistochemically defined Luminal A and Luminal B/HER2 negative tumors and were mostly towards ACT.

Conclusions: Omission of AD in patients with a positive SLN receiving BCS would have altered the indication to ACT in 16% of the patients at our Institution. Changes occurred almost exclusively in patients with hormone receptor positive/HER2-negative tumors. The implications of omission of AD must be taken into account before its widespread acceptance, including the possibility of a biologically tailored surgical approach.

P3-07-05
Nomogram Incorporating SLN Metastasis Size Provides the Most Accurate Prediction of Non-SLN Involvement in Breast Cancer Patients.
Gainer SM, Mittendorf EA, Hunt KK, Bassett RL, Yi M, Meric-Bernstam F, Ross MI, Babiera GV, Kuerer HM, Candle AS, Hwang RF, UT-M.D. Anderson Cancer Center, Houston, TX

Introduction: The finding of metastatic disease in the axillary nodes remains an important factor that guides adjuvant therapy decisions in breast cancer. The value of completion axillary lymph node dissection (ALND) may not be the same in all patients and determining the risk of non-SLN involvement can be a guide in surgical decision making. We recently developed a nomogram that incorporates the maximum SLN metastasis size to predict non-SLN axillary involvement (Mittendorf et al., Ann Surgery 2011, in press). The purpose of this study was to compare the accuracy of this nomogram to another widely used nomogram that does not include SLN metastasis size.

Methods: We queried a prospective breast cancer database for patients with clinically negative nodes undergoing sentinel node biopsy who did not receive neoadjuvant therapy. We identified 431 patients with invasive breast cancer treated from 1996 to 2007 who had a positive SLN and underwent completion ALND. We evaluated clinicopathologic data including histology, multifocality, nuclear grade, pathologic tumor size, number of SLNs recovered, number of positive SLNs, maximum SLN metastasis size, presence of extranodal extension, hormone receptor status, and presence of lymphovascular invasion. Data were entered into our nomogram and the nomogram that does not include SLN metastasis size. The accuracy of the 2 nomograms in predicting non-SLN axillary metastasis was compared in all patients and in subgroups of patients based on burden of disease in the SLN.

Results: For the entire cohort of 431 women with positive SLNs, our SLN size nomogram was more accurate in the detection of non-SLN disease (area under the receiver operating characteristic curve [AUC] 78.3% vs. 72.4%, p<0.01). Whether or not SLNs were subjected to frozen section did not influence the relative accuracies of the 2 nomograms. When patients were analyzed by burden of disease in the SLNs, our SLN size nomogram was more accurate in predicting non-SLN disease in patients with macrometastases in the SLN (AUC 77.3% vs. 70.3%, p=0.008). There was no significant difference in the accuracy of the 2 nomograms for patients with isolated tumor cells or micrometastases in the SLNs.

Conclusions: Our nomogram incorporating SLN metastasis size with other clinicopathologic factors is the most accurate predictor of the risk of non-SLN metastasis in patients with a positive SLN. The SLN size nomogram is highly accurate in women with macrometastases, who may benefit the most from completion ALND. Using a nomogram with SLN metastasis size can provide the most accurate information for patient counseling and surgical decision making.
P3-07-06
Prognostic Utility of Upfront Nodal Staging Prior to Neoadjuvant Chemotherapy: The UAB Experience.
Purpose: Controversy exists regarding the prognostic utility of upfront lymph node staging in patients receiving neoadjuvant chemotherapy. This retrospective study explores whether upfront versus outback nodal staging influenced locoregional control and survival endpoints.
Methods: Between 1999 and 2005 one hundred and fifteen patients treated with neoadjuvant chemotherapy at UAB were identified. Patient, tumor, and treatment variables were recorded. Timing of nodal assessment was based on either surgeon preference or stipulated upfront per several clinical protocols. Survival was measured using the Kaplan Meier statistics. Univariate and multivariate analyses of covariates associated with local-regional control (LRC), progression-free (PFS) and overall survival (OS) were performed. Results: Mean age was 49 years and mean follow-up was 5.8 years. Stage distribution was as follows: 40 IIA, 34 IIB, 26 IIIA, 10 IIIB, and 5 IIIC. Definitive surgery included breast conservation in 49 patients, total mastectomy in 21 and modified radical mastectomy in 44. Seventy-two patients had upfront nodal sampling before neoadjuvant therapy, 36 by fine needle assessment and the remainder by sentinel node biopsy. Forty-three patients had their nodal assessment following neoadjuvant chemotherapy. Of those with upfront nodal staging: forty-nine patients had a positive nodal result and 23 had negative findings. In those that were sampled at the time of definitive surgery: 21 had positive results, 21 had a negative result, and 1 patient did not have any nodes in the specimen. One hundred five patients had post-operative radiation therapy. Overall there was no difference in LRC, PFS or OS outcomes between patients that had an upfront nodal staging procedure and those that had their nodes sampled at the time of definitive surgery. Patients achieving pCR had a non-significant trend towards improved overall survival (p=0.12). Final pathologic T, N and group stage were statistically significant in determining the progression free, p=0.003, 0.011, 0.005 and overall survival, p=0.02, 0.037, and 0.009. Lymphovascular space invasion was associated with progression free survival on univariate analysis, p=0.0179, but not multivariate analysis. Other covariates including age, biologic subtype, grade, type of chemotherapy, use of radiation therapy, radiation volume, time to complete radiation therapy, and use of hormonal therapy did not affect outcomes. Discussion: Upfront as compared with outback lymph node staging did not influence locoregional control or survival endpoints. Final pathologic stage and not initial clinical stage remains the most important prognostic factor associated with survival.

Accuracy of 2 nomograms in predicting axillary metastasis in non-SLNs in breast cancer patients with positive SLNs

<table>
<thead>
<tr>
<th>SLN metastasis size</th>
<th>Isolated tumor cells (≤0.2 mm) (n=13)</th>
<th>Micrometastases (0.2-2 mm) (n=112)</th>
<th>Macrometastases (≥2 mm) (n=306)</th>
<th>Overall (n=431)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC for nomogram incorporating SLN metastasis size</td>
<td>77.3%</td>
<td>73.3%</td>
<td>77.3%</td>
<td>78.3%</td>
</tr>
<tr>
<td>AUC for nomogram not incorporating SLN metastasis size</td>
<td>100%</td>
<td>74.7%</td>
<td>70.1%</td>
<td>72.3%</td>
</tr>
<tr>
<td>p-value</td>
<td>0.34</td>
<td>0.79</td>
<td>0.008</td>
<td>0.01</td>
</tr>
</tbody>
</table>

P3-07-07
Effect of Sentinel Lymph Node Biopsy without Axillary Lymph Node Dissection on Overall Survival in Patients with T1 or T2 Node-Positive Breast Cancer: A Report from the Korean Breast Cancer Society.
Park HS, Chae BJ, Song BJ, Jung SS, KBCS Investigators. The Catholic University of Korea College of Medicine, Seoul, Korea; Korean Breast Cancer Society
Introduction: Sentinel lymph node biopsy (SLNB) is accepted as an alternative method to axillary lymph node dissection for staging axillary lymph node status in clinically node-negative breast cancer. Current practical guidelines recommend that axillary lymph node dissection (ALND) should be performed in cases with sentinel node metastasis, and most of node-positive patients should receive adjuvant systemic therapy to reduce locoregional/distant recurrence and to improve overall survival irrespective of the number of lymph node metastasis. However, patients with ALND are more likely to develop lymphedema than those with SLNB alone, and appropriate systemic chemotherapy or hormone therapy significantly reduce locoregional and distant recurrence in early breast cancer patients. For this reason, the previous prospective study, American College of Surgeon Oncology Group Z0011 trial, was conducted and it suggested that there is no difference in overall survival between node-positive patients who received breast conserving treatment with SLNB alone and those with ALND after SLNB. This study is aimed to evaluate the difference of survival between node-positive patients who underwent SLNB alone and those who received ALND after SLNB using the Korean Breast Cancer Society registry.
Methods: In 87671 patients with breast cancer in the registry, we enrolled 2581 patients who meet the eligible criteria in the study. All enrolled patients had T1 or T2 breast cancer, and received mastectomy or breast conserving treatment followed by documented adjuvant systemic therapy between Jan. 2001 and Apr. 2011. Log-rank test and Cox-proportional hazard model were used to access the difference of overall survival according to the axillary procedure.
Results: There were 197 patients with SLNB alone and 2384 patients with ALND after SLNB, respectively. Smaller tumor size, lower number of nodal metastasis, and higher proportion of breast conserving surgery were shown in patients with SLNB alone than in those with ALND after SLNB. There was no significant difference in overall survival between 2 groups in the log-rank test. ALND after SLNB showed no significant improvement on overall survival in Cox-proportional hazard model adjusted by tumor size, number of nodal metastasis, and operation type (P=0.78, HR=0.73, 95% CI=0.80-6.62). Conclusion: The current study suggests that ALND after SLNB in cases with sentinel lymph node metastasis may not influence on the improvement of overall survival and supports the results of Z0011 trial. Further validation studies are necessary to expand the understanding of the role of performing SLNB alone in patients with node-positive breast cancer.

P3-07-08
Accurate Staging of Axillary Lymph Nodes from Breast Cancer Patients Using a Novel Molecular Method.
Okato T, Iwase T, Kimura K, Yamashita K, Horii R, Akiyama F. Cancer Institute Hospital of Japanese Foundation for Cancer Research, Koto-ku, Tokyo, Japan
Background: In breast cancer, the number of axillary lymph node metastases is the powerful prognostic factor. However, it is obvious that conventional histopathological examinations are non-standardized...
and limited in their ability to detect metastases accurately due to the partial evaluation of a node. This may lead to underestimation of nodal staging. The one-step nucleic acid amplification (OSNA) assay was developed to overcome this limitation of the histopathological examination. This assay can assess the whole lymph node and yields semi-quantitative results for the detection of clinically relevant nodal metastases by detection and amplification of cytokeratin 19 mRNA. This assay can classify the nodes into 4 categories, (+), (++), (+I), (+), and negative. (+++) and (+I) are theoretically regarded as macrometastasis and (+) as micrometastasis according to the American Joint Committee on Cancer (AJCC) staging system. We have shown the OSNA whole node assay detects more sentinel node (SN) metastases, particularly micrometastases than 2-mm-section frozen-section histology. Thus, we had hypothesized that the OSNA assay for non-sentinel nodes (nonSNs) in addition to SNs enables the classification of accurate nodal staging for breast cancer patients. In the present retrospective cohort study, we compared the performance of the OSNA assay with that of routine permanent histology for the detection of nonSN metastases among patients with positive SN biopsy who have undergone axillary dissection.

Patients and methods: Subjects comprised of consecutive 183 patients with clinically and ultrasonographically node-negative pT1-2 breast cancer who had undergone axillary dissection after positive SN biopsy with the OSNA assay between April 2009 and September 2010. Of these, for nonSN evaluation, 64 had single-section permanent histology while 119 patients underwent the OSNA whole node assay. We compared 1) detection rates of nonSN metastasis, including macro- and micrometastases and 2) upstaging rates from SN stage after the nonSN assessment according to the 7th AJCC staging system between both cohorts. We performed the two-population z test.

Results:
1) NonSNs were found to be positive for metastasis more frequently in the OSNA cohort than in the histology cohort (histology 13/64, 20.3%, 95% CI 11.7-32.6% vs. OSNA 66/119, 55.5%, 95% CI 46.1-64.5%; P<0.001). We found no significant difference in the frequency of macrometastasis in nonSNs (12/64, 18.8%, 95% CI 10.5-30.8% vs. 30/119, 25.2%, 95% CI 17.9-34.2%; P=0.42). However, we found significant difference in the frequency of micrometastasis in nonSNs (1/64, 1.6%, 95% CI; 0.1-9.5% vs. 36/119, 30.3%, 95% CI; 22.3-39.5%; P=0.001).

2) Total upstaging rates were similar in both cohorts (histology 9/64, 14.1%, 95% CI 7.0-25.5% vs. OSNA 20/119, 16.8%, 95% CI 10.8-25.0%; P=0.79).

Conclusion: The OSNA whole node assay detects a far greater proportion of nonSN metastases than single-section histology in patients with positive SN biopsy. However, in terms of the AJCC staging system, upstaging rates from the SN stage were similar in both cohorts. Follow-up of the OSNA cohort is required to clarify the prognostic implications of this technique; this may lead to the establishment of a new breast cancer staging.

P3-07-09


Background: The Memorial Sloan Kettering Cancer Center-Breast Cancer Nomogram (MSKCC-BCN) predicts additional nodal metastasis in patients with positive sentinel lymph node (SLN). This statistical tool does not include HER2 status. It has been shown that the interaction covariate between estrogen receptor (ER) and HER2 status was a determinant of SLN positivity. The purpose of our study was to determine if the accuracy of MSKCC BCN could be enhanced with new variables.

Patients and methods: Our dataset consisted of 2769 consecutive patients treated for operable breast cancer with SLN biopsies between 2006 and 2009. We selected all the patients (n = 588) with a positive SLN who underwent a completion axillary lymph node dissection (ALND). The MSKCC-BCN was used to calculate the theoretical risk of additional nodal metastasis for all patients. The evaluation of the MSKCC-BCN was performed with calibration test (Cox method) and performance test (Bleeker). Multivariate analysis used a logistic regression model. The input was based on the variables found significant in the univariate analysis. Interaction covariate between ER and HER2 status was included in the model. Our model was then analyzed in terms of discrimination (area under the curve) and of calibration (Hosmer-Lemeshow test).

Results: Calibration test showed significant differences between the probability of additional nodal disease predicted by the MSKCC-BCN and the probability observed in our population for the following subgroups of patients: histological grade 3 (p = 0.007), lymphovascular invasion (p = 0.03), multifocality (p = 0.04), positive ER (p = 0.002), micrometastasis in the SLN (p = 0.003), isolated tumor cells in the SLN (p = 0.02 and positive HER2 (p = 0.01). Performance test showed significant differences for the following variables: histological grade (p = 0.02), size of the SLN metastasis (p = 0.04) and HER2 status (p = 0.01). This shows that the MSKCC-BCN is not well calibrated and cannot be used for our population. A multivariate model to determine the probability of additional nodal metastasis was defined with the following variables: pathologic size of the sentinel node metastasis, interaction covariate between the ER and HER2 status, number of positive SLN and number of SLN removed [Table 1].

Table 1: Multivariable analysis

<table>
<thead>
<tr>
<th>Significant variable</th>
<th>OR</th>
<th>CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Micrometastasis</td>
<td>0.15</td>
<td>0.08-0.28</td>
<td>0.0001</td>
</tr>
<tr>
<td>Isolated tumor cells</td>
<td>0.16</td>
<td>0.08-0.32</td>
<td>0.0001</td>
</tr>
<tr>
<td>ER-HER2-</td>
<td>2.97</td>
<td>2.25-7.76</td>
<td>0.003</td>
</tr>
<tr>
<td>ER-HER2+</td>
<td>5.99</td>
<td>0.76-47.25</td>
<td>0.002</td>
</tr>
<tr>
<td>ER-HER2</td>
<td>3.47</td>
<td>1.48-8.76</td>
<td>0.003</td>
</tr>
<tr>
<td>N positive SLN</td>
<td>1.93</td>
<td>1.92-2.82</td>
<td>0.0006</td>
</tr>
<tr>
<td>N negative SLN</td>
<td>0.74</td>
<td>0.64-0.85</td>
<td>0.0003</td>
</tr>
</tbody>
</table>

This multivariate model resulted in a nomogram tested on the training population. It was discriminating with an area under the curve of 0.76 [0.720-0.808] and well calibrated (Hosmer-Lemeshow test p = 0.51).

Conclusions: We showed that HER2 status and pathologic size of the SLN metastasis are determinant to predict additional nodal metastasis after a positive SLN. These two variables were included in a new nomogram that could help in the decision-making concerning further axillary treatment in these patients. Our model has to be validated prospectively in external series to confirm its accuracy.

P3-07-10
Sentinel Lymph Node Navigation Surgery Using Combination of Indocyanine Green Fluorescence and Blue Dye for Breast Cancer Patients.

Takahashi M, Hayashida T, Jinno H, Kitagawa Y. Keio University School of Medicine, Tokyo, Shinjuku, Japan

Background: Sentinel lymph node (SLN) biopsy is now widely accepted for staging the axilla in breast cancer patients. Several methods are presently used to detect SLNs. Combined use of blue dye and radiocolloid has been previously described as a superior method for detecting SLNs in breast cancer patients, but radiocolloid is not...
generally available in Japan. SLN detection guided by indocyanine green (ICG) fluorescence could emerge as a new and effective method for SLN biopsy. The aim of this study was to evaluate SLN biopsy using combination of ICG fluorescence and blue dye methods for breast cancer patients.

Material and Methods: We enrolled 75 patients with clinically node-negative breast cancer. An isosulfan blue dye solution (5ml) was injected subcutaneously just above the tumor and in the periareolar area after the induction of anesthesia. The injection site was massaged for five minutes and ICG (2ml) was then injected subcutaneously just above the tumor and in the periareolar area before starting the surgical procedure. Subcutaneous lymphatic channels draining from the areola to the axilla were visualized with a fluorescence imaging system (Photodynamic Eye: PDE, Hamamatsu Photonics Corporation, Japan) under fluorescence within several minutes. SLNs were dissected under direct visualization supported by fluorescence and dye navigation and diagnosed by intraoperative frozen section with hematoxylin and eosin staining. Any patient with a positive SLN underwent immediate axillary lymph node dissection (ALND). Results: SLNs were detected in all 75 patients. The fluorescence imaging identified 2.6 SLNs in 74 (98.7%) of 75 patients. The blue dye identified 1.6 SLNs in 68 (90.1%) of 75 patients. Twelve of the 75 (16.0%) patients had metastatic SLNs and ALND was immediately performed. No metastasis revealed in any non-SLN in eight of these 12 patients. Metastatic SLNs were detected by the ICG fluorescence and not by blue dye in four of these 12 patients.

Discussion: The combination of ICG fluorescence and blue dye method had two advantages; it could reveal lymph flow on the surface before we determined the skin incision line; and it had a high SLN detection rate. In hospitals where radioisotopes are unavailable, a combination of the two methods would be advantageous.

Mean Number of Sentinel Lymph Nodes and Detection Rate

<table>
<thead>
<tr>
<th>Detection Method</th>
<th>Mean Number</th>
<th>Detection Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dye-Positive</td>
<td>2.6</td>
<td>67/75 (89.3%)</td>
</tr>
<tr>
<td>Fluorescence-Positive</td>
<td>2.6</td>
<td>75/75 (100%)</td>
</tr>
<tr>
<td>Combined Method</td>
<td>2.6</td>
<td>75/75 (100%)</td>
</tr>
</tbody>
</table>

A median of 1.95 SLNs were assessed by FS and a median of 2.06 SLNs by OSNA (p=0.15). Intraoperative histopathological assessment detected 67 SLNs metastasis by FS and 107 SLNs metastasis by OSNA assay (p=0.07). There were no differences in rates of macrometastasis (54 by FS and 74 by OSNA) and micrometastasis (13 by FS and 33 by OSNA) (p=0.09). When compared the OSNA assay with permanent section, 92 SLNs had metastasis by permanent section (63 macrometastasis and 29 micrometastasis) with no statistically significant differences (p=0.5).

SLNs metastasis were found in 71 patients (31.1%) by permanent section and in 83 (33.2%) by OSNA assay (p=0.69).

Axillary lymph node dissection (ALND) for metastatic SLN was performed in 148 patients, 79 (31.6%) in OSNA group and 69 (30.2%) in the permanent section group (p=0.68). All patients diagnosed by OSNA had a complete ALND during the initial surgical procedure. On the other hand, ALND was performed in 51 patients (73.9%) in the permanent section in the initial surgery, and ALND was performed in a second surgical procedure in 18 patients (26%), due to false negative results of the FS (p<0.001).

CONCLUSIONS:
The OSNA assay can detect SLN metastasis as accurately as conventional pathology, with no increased detection of positive SLNs. Given the definitive pathology of the SLN intraoperatively, the use of OSNA can reduce the need for a second surgery in 26% of patients with breast cancer and a positive SLN.

P3-07-12


Diepstraten SC, Sever AR, Buckens CF, Veldhuis WB, van den Bosch MA, Mali WP, Verkooijen HM. University Medical Center Utrecht, Netherlands; Maidstone Hospital, United Kingdom

Purpose: Women affected with invasive breast cancer generally undergo sentinel node biopsy (SNB) of the axilla, which is followed by axillary dissection if lymph node metastases are found. This systematic review and meta-analysis aims to evaluate the utility of...
preoperative axillary ultrasound-guided needle biopsy in terms of staging the axilla and preventing futile SNB.

Methods: We systematically searched Pubmed, Embase and Cochrane through March 14, 2011, for studies addressing preoperative assessment of axillary lymph node (ALN) status by axillary ultrasound and biopsy. Eligible studies had to report on biopsy indication and method, and true and false positive and negative outcomes. A pooled estimate was calculated for prevalence of futile SNB (defined as the proportion of patients needing secondary ALN dissection after a negative axillary ultrasound and biopsy) and sensitivity (defined as the proportion of patients with ALN involvement detected by axillary ultrasound and biopsy).

Results: Twenty-three studies were included which reported on a total of 6205 procedures. The pooled prevalence of futile SNB was 25% (95% CI = 23% to 27%) and the pooled sensitivity was 51% (95% CI = 43% to 59%). There was substantial heterogeneity across studies for both futile SNB prevalence (I² = 60.5) and sensitivity (I² = 93.3), which could not be explained by between-study differences in biopsy technique, inclusion criteria, biopsy indication or study design. Sensitivity was increased in studies with a high prevalence of axillary lymph node metastases.

Conclusion: Preoperative axillary ultrasound guided biopsy is a useful step in the process of axillary staging as around fifty percent of breast cancer patients with axillary involvement can be identified pre-operatively and are spared a two step surgical intervention. Still, one in four women with a US biopsy ‘proven’ negative axilla have ALN involvement on SNB and need to undergo completion axillary dissection. New techniques may be able to reduce the prevalence of futile SNB.


Chen K, Jia W, Zeng Y, Fan M, Su F, Li S. Sun Yat-sen Memorial Hospital, Sun Yat-sen University, Guangzhou, Guangdong, China

Background: Several models for predicting the risk of nonsentinel lymph node (NSLN) metastasis in breast cancer patients with positive sentinel lymph nodes (SLNs) have been developed. Independent validation of these models in different populations is necessary before clinical application. This study aimed to validate and compare these models in Chinese patients.

Patients and Methods: A total of 159 breast cancer patients with positive SLNs treated at our institution were included. Chi-squared tests, RP-ROC, CART and multivariate logistic regression were used to analyze the risk factors for NSLN involvement in our population. ROC curves, calibration plots and false-negative (FN) rates were evaluated for 11 reported models. The threshold of each model for classifying patients into the low-risk group was adjusted to render the FN rate close to 10%.

Results: In total, 81 (50.9%) patients had at least one NSLN involvement. Univariate and multivariate analyses revealed that the number of negative SLNs (P=0.01, HR=0.63) and the metastasis size of the positive SLNs (P=0.01, HR=1.15) independently predicted the NSLN status in our population. The Cambridge and Mou models outperformed the others, both with AUCs of 0.73. The other models performed as follows: the Mayo, Tenon, MDA, MSKCC, Ljubljana, SNUH and Louisville models had AUCs of 0.68, 0.66, 0.66, 0.64, 0.62, 0.61 and 0.60, respectively. The Stanford and Saidi models did not present any discriminative capabilities, with AUCs of 0.54 and 0.50, respectively. The Cambridge, MSKCC and Mayo models were well calibrated. The Ljubljana model did not calibrate well.

With adjusted thresholds, the Mayo model outperformed the others by classifying the highest proportion of patients (20%) into the low-risk group. The Cambridge, Mou and MDA models defined 17.0%, 14.5% and 15.1% of patients as low-risk, respectively.

Sensory Disturbance of the Ipsilateral Upper Arm after Breast Cancer Surgery with Sentinel Node Biopsy Alone Compared with Axillary Dissection – A Prospective Study.

Okusumi S, Kiyoto S, Takahashi M, Haru F, Takabatake D, Takashima S, Aogi K, Shimozuma K. NHO Shikoku Cancer Center, Matsuyama, Ehime, Japan; Ritsumeikan University, Kusatsu, Shiga, Japan

Background: Axillary surgery for breast cancer causes several postoperative complications including edema of the ipsilateral arm and sensory disturbance of the ipsilateral upper arm. Although sentinel node biopsy has been considered as a standard procedure for node-negative patients, some complications bother patients even after it. Although data regarding the quality of life of patients and/or subjective and objective assessment of arm morbidity after axillary surgery have been reported, to our knowledge, quite a few data have been reported on actual examination of sensory disturbance of the ipsilateral upper arm after sentinel node biopsy alone. We report comparative data regarding the objective and subjective degrees of postoperative sensory disturbance of the ipsilateral upper arm examined prospectively between sentinel node biopsy alone and axillary dissection.

Patients and Methods: A total of 118 patients, who received breast cancer surgery with sentinel node biopsy alone (51 patients)(SN group) or axillary dissection (67 patients)(AD group) at NHO Shikoku Cancer Center, were prospectively evaluated sensory disturbance regarding the following: (a) dysesthesia, (b) paresthesia, (c) degree of disturbed tactile sensation, (d) degree of disturbed pain sensation, (e) ratio of disturbed area of tactile sensation (defined as A/B) length of disturbed area (B) total length of upper arm, and (f) ratio of disturbed area of pain sensation (defined as A/B). Patients were either asked
about the above or examined for them at one month, 6 months and 1 year, respectively, after surgery. At surgery it was recorded whether or not the intercostobrachial nerve(s) were totally preserved in patients who underwent axillary dissection. Unpaired t-test, Mann-Whitney U-test and Wilcoxon signed rank test were used to test statistical significance.

Results: The mean ages at surgery were 54.7 and 53.7 years in the SN and AD groups, respectively (P = 0.66). The mean number of biopsied lymph nodes in the SN group was 2.4 (range: 1-5). In 22 patients of the AD group the intercostobrachial nerves were totally preserved. There was no difference in dysesthesia, paresthesia, and the level of disturbed pain sensation between the two groups throughout one year after surgery. However, the patients in the SN group showed milder disturbed tactile sensation at one and 6 months than those in the AD group (P = 0.04 and 0.03, respectively). Both the ratios of disturbed area of tactile and pain sensation were statistically significantly lower in the SN group than in the AD group throughout one year (P = 0.03 ~ <0.0001). The mean ratios of disturbed area of those sensations were stable in the SN group over the year (0.03-0.05 for both tactile and pain sensations, P > 0.12 for any comparison). On the other hand, the mean ratios in the AD group became lower from 0.23 to 0.17 for tactile sensation and from 0.20 to 0.13 in pain sensation (P = 0.02, and 0.05 for tactile and pain sensations, respectively).

Conclusion: Sensory disturbance of the ipsilateral upper arm after sentinel node biopsy alone was much milder than after axillary dissection throughout the study period.

P3-07-15
Sequential Peri-Areolar and Peri-Tumoural SPECT/CT Lymphoscintigraphy Has Identified High Rates of Discordance in Both Axillary and Internal Mammary Sentinel Lymph Node Mapping.
Nouthis F, Spillane A, Gehski V, Snook K, Gillet D, Cooper R, Allwright S, Uren R, Sydney University, Sydney, New South Wales, Australia; Mater Hospital, Crows Nest, New South Wales, Australia

Introduction:
The release of the MA20 intergroup trial results has confirmed the importance of locoregional radiation on local and distant disease control and survival. Sentinel lymph node (SLN) evaluation is the gold standard for regional lymph node evaluation and radiation oncologists continue to utilize the nodal staging to plan adjuvant locoregional therapy. Despite over a decade of experience in SLN evaluation there persists a common belief that the lymphatic drainage of the whole breast is to anteroposterior axillary lymph nodes. Several different lymphoscintigraphy injection techniques are in use with claims that they all identify the same axillary sentinel nodes. However this is not based on a high level of evidence. The evolution of SPECT/CT has led to the accurate anatomic identification of SLN, hence the different lymphoscintigraphy techniques can now be directly compared.

Method:
38 patients underwent double sequential lymphoscintigraphy (periareolar followed by peri-tumoural) separated by 1-7 days. Patients were referred by 4 surgeons to 3 separate lymphoscintigraphy centres with standardisation of tracer substance (99mTc-antimony sulfide colloid), lymphoscintigraphy and SPECT/CT evaluation techniques. The degree of discordance in sentinel node evaluation was defined as:

• Type 1 - One study demonstrates SLN(s) in a nodal basin [axilla or internal mammary chain (IMC)] and the other study demonstrates none.
• Type 2 - Both studies identified SLN(s) in a nodal basin however the SLN(s) are all different.

• Type 3 - Both studies identified SLNs in a nodal basin, however some SLN(s) are identical and others not.

Notably 21 patients (55%) had either axilla or IMC lymphoscintigraphy discordance. Two patients had both axilla and IMC lymphoscintigraphy discordance.

The majority of discordance was identified in the IMC. In the discordant IMC lymphoscintigraphies neither study demonstrated IMC SLN. There were no studies that identified identical IMC nodes in both PA and PT lymphoscintigraphy. Discordant axillary drainage was more evident in patients with lateral sector tumours compared to IM discordance with medial sector tumours.

Conclusions:
Accurate lymphoscintigraphy is the road map to assist a surgeon identify lymph node metastases. In turn accurate staging will determine appropriate adjuvant therapy decisions particularly regional node irradiation. This study demonstrates a high degree of discordance between PA and PT lymphoscintigraphy techniques in 55% of patients. Inadequate lymphoscintigraphy can result in unrealized false negative results, which could impact patient outcomes and clinical trial results.

P3-07-16
Seo YJ, Hwang MJ, Lee JH, Son GT, Choi JE, Bae YK, Kang SH, Lee SJ, Yeungnam University College of Medicine, Daegu, Republic of Korea

Background:
Lymphedema affects over 20% of breast cancer patients undergoing axillary dissection. Axillary reverse mapping (ARM) technique to identify and preserve arm node during sentinel lymph node biopsy (SLNB) or axillary lymph node dissection (ALND) was developed to prevent lymphedema. The purpose of this study was to investigate the location and the metastatic rate of the arm node, and finally to evaluate the short term incidence of lymphedema after arm node preserving surgery.

Patients and Methods:
From January 2009 to October 2010, 97 breast cancer patients who underwent ARM were enrolled. 2.5ml blue dye was injected in ipsilateral upper inner arm. After at least 20 minutes after injection, SLNB or ALND was performed in the usual manner and blue stained arm nodes and/or lymphatics were identified. We checked arm circumference at baseline and average of 8.8 months after operation in ALND cases and 13.7 months in SLNB cases. Patients were divided into two groups, arm node preserved group (70 patients in ALND, 10 patients in SLNB) and unopened group (13 patients in ALND, 4 patients in SLNB). The difference of arm circumference between preoperative and postoperative was checked in these groups.

Results:
The mean number of identified blue stained arm nodes was 1.4±0.6. The arm nodes were found in the inferolateral side of axillary and thoracodorsal vessels in 57 patients (58.76%), the inferomedial side in 37 patients (38.14%), the superolateral side in 2 patients (2.06%), and the superomedial side in 1 patient (1.04%). In the majority of patients (92%), arm nodes were located between the lower level of the axillary vein and just below the second intercostal brachial nerve.
In arm node unpreserved group, 2 patients had metastasis in their arm node. The one had a common pathway between the arm node and the sentinel lymph node. Another did not have a common pathway, but had extranodal extension with N3 metastasis. Among ALND patients, in arm node preserved group, the difference of arm circumference between preoperative and postoperative in ipsilateral and contralateral arm was 0.27cm and 0.07cm, respectively, whereas 0.47cm and -0.03cm in unpreserved group, and one lymphedema was found after 6 months. No difference was found between arm node preserved and unpreserved group among SLNB patients (0.21cm and 0.39cm in in preserved group, 0.2cm and 0.02cm in unpreserved group).

**Conclusion:** Arm node preserving was possible in all breast cancer patients with identifiable arm node, during ALND or SLNB, except for those with high surgical N stage, and lymphedema did not develop in patient with arm node preserving surgery. Metastasis was not found in arm node preserving group in current results, but need to be observed in the ongoing progress.

**P3-07-17**

**Optimal Number of Sentinel Lymph Nodes That Could Avoid Completion of Axillary Lymph Node Dissection in Operable Breast Cancer.**

Salmon RJ, de Ricke Y, Falcon MC. Institut Curie, Paris, France

**Background:** Axillary lymph node dissection (ALND) has been the standard of care for breast cancer patients with sentinel lymph node (SLN) metastasis. It is now under discussion since the publications of ACOZOG Z0011 and NSABP-32. The 1-3% rate of axillary recurrence is very low in these studies, whereas the rate of additional nodal metastasis after the completion of ALND varies from 15% to 35%. The purpose of this study was to determine the optimal number of nodes to be removed in order to obtain an axilla free of disease after initial surgery.

**Patients and methods:** 4928 consecutive patients treated for breast cancer ≤ 2 cm with primary breast surgery with SNB between 2000 and 2009 were reviewed from the Institut Curie breast cancer prospective database. 1165 patients with a positive SLN (macrometastasis, micrometastasis and isolated tumor cells) underwent ALND. The proportion of patients with positive ALND was analysed according to the total number of sentinel lymph nodes biopsied. Results were compared with Chi-square test for qualitative variables and with Kruskal-Wallis for quantitative variables.

**Results:** Among the 1165 patients, 308 (26.4%) had a positive ALND. Among them, 81.1% of patients had a macrometastasis in the SLN versus 45.6% in the 857 patients with negative ALND (p < 0.0001); 10.8% had a micrometastasis versus 31.4% (p < 0.001) and 8.1% of patients had isolated tumor cells in the SLN versus 23% (p < 0.001). The proportion of patients with positive ALND decreased with the number of sentinel nodes removed (Figure 1), independently of the number of positive SLN. Among the 1165 patients, the rate of patients with 5 nodes removed or more and with residual disease in the axilla was < 1% (n=11). Those patients could have avoided completion ALND, representing 3.6% of the total positive ALND that would have been neglected.

**Conclusions:** We showed that patients with positive SLN have a very low risk (<1%) of residual disease in the axilla when 5 nodes or more have been removed. This number which is a major component of nomograms, could be used as a threshold to avoid completion ALND.

**P3-07-18**

Withdrawn by Author

**P3-07-19**

**Long-Term Outcome of Internal Mammary Lymph Node Detected by Lymphoscintigraphy in Early Breast Cancer.**


**Purpose:** The purpose of this study is to determine the long-term significance of internal mammary lymph node (IMLN) detected by lymphoscintigraphy.

**Background:** IMLN metastasis is an important prognostic indicator in breast cancer. However, the necessity of internal mammary sentinel lymph node (IMSLN) biopsy for accurate staging, for choosing adjuvant treatment, and as a prognostic indicator, has remained controversial.

**Methods:** From January 2001 until December 2006, 525 female breast cancer patients underwent radical surgery after preoperative lymphatic scintigraphy. We retrospectively analyzed the follow-up results, recurrences and deaths of all patients.

**Results:** There was no significant difference in clinicopathologic characteristics between the axilla and the IMLN group. The median follow-up period was 118.8 (range, 7-122) months in the axilla group and 107.7 (range, 14-108) months in the IMLN group. During the median follow-up period, the breast cancer-related death rate in the axilla group was 3.6%, which was not significantly different from that of the IMLN group (1.3%) (P = 0.484). The five-year survival rates (5YRS) did not differ between the two groups (P = 0.306). The overall recurrence rate and the locoregional (LR) recurrence rate also did not differ between the two groups (P = 0.835 and P = 0.582, respectively). The recurrence rate of IMLN (both ipsilateral and contralateral) metastasis was very low, accounting for only 0.5% in the axilla group and 1.3% in the IMLN group (P = 0.416).**Conclusion:** The long-term follow up results showed that there was no significant difference in both overall outcome and regional recurrence between the two groups. Therefore, the necessity of identification of nodal basins outside the axilla or the necessity of IMLN sentinel biopsy should be reconsidered.

**P3-07-20**

**An Independent Assessment of Seven Nomograms for Predicting the Probability of Additional Axillary Nodal Metastasis after Positive Sentinel Lymph Node Biopsy in a Cohort of British Breast Cancer Population.**

Nadeem RM, Gudur LD, Saidan ZA. Lancashire Teaching Hospitals NHS Foundation Trust, Chorley, United Kingdom

**Background:** Axillary lymph node dissection (ALND) is currently the recommended procedure in patients with positive sentinel lymph node (SLN) biopsy. A significant proportion of patients with positive SLNs will not have any additional metastasis in non-sentinel lymph nodes (NSLNs). Nomograms could identify a subgroup of patients with low risk of further disease in whom completion ALND can be avoided. The aim of this study is to assess the accuracy of currently available seven nomograms in a cohort of British breast cancer population.
Methods: 138 patients with positive SLNs who underwent completion ALND between October 2006-April 2011 were identified. Multiple pathological variables including the histological size of the SLNs metastasis were analysed. Data was then used to calculate the probability of further metastasis in non-SLNs according to the seven nomograms that are currently in use: MSKCC, Cambridge, Turkish, Stanford, MDACC, Tenon and MOU models. The area under the receiver-operator characteristic (ROC) curve (AUC), 95% confidence intervals, false negative and positive rate were calculated for each nomogram. Subgroup analysis includes pathological tumour size (≤ 2 cm and >2 cm) and macro- or micrometastasis in SLNs. AUC value ≥ 0.80 has an excellent discriminatory power.

Results: 56 of 138 patients (41%) had additional metastasis in non-SLNs.

AUC values with 95% confidence intervals and false negative rate are shown in table 1.

<table>
<thead>
<tr>
<th>Nomograms</th>
<th>MSKCC</th>
<th>Cambridge</th>
<th>Turkish</th>
<th>Stanford</th>
<th>MDACC</th>
<th>Tenon</th>
<th>MOU</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC values</td>
<td>0.68</td>
<td>0.68</td>
<td>0.70</td>
<td>0.69</td>
<td>0.56</td>
<td>0.63</td>
<td>0.74</td>
</tr>
<tr>
<td>95% CI</td>
<td>0.59-0.78</td>
<td>0.58-0.77</td>
<td>0.61-0.79</td>
<td>0.60-0.78</td>
<td>0.43-0.69</td>
<td>0.54-0.72</td>
<td>0.65-0.82</td>
</tr>
</tbody>
</table>

Subgroup analysis also shows persistently better AUC value for MOU nomogram. None of the models were able to achieve an AUC value > 0.80 in a cohort of British breast cancer patients.

Discriminative power.

Conclusions: 46/138(33.3%) had tumour size ≤ 2 cm and 18/46(39%) had additional NSLNs metastasis. AUC values were calculated for each nomogram. Subgroup analysis includes the following variables: tumour size (≤ 2 cm and >2 cm) and macro- or micrometastasis in SLNs.

Discussion:

Conclusion:

Methods: A total of 497 patients with clinically node negative T1-T3 breast cancer underwent SLN biopsy from 2005 to 2009. Patients were divided in two groups depending on the timing of SLN biopsy. Patients were classified according to ER/PR/Her2 status as (ER/PR/HER2 + (TP), ER/PR/HER2 – (TN), ER/PR + HER2 - and ER/PR -HER2 +). For SLN metastasis the following variables were tested in univariable and multivariable models: age, tumor grade, tumor type, and the combined hormone receptors and Her2.

Results: The SLN was positive in the surgery first group in 127 of 429 patients (29.6%) while in 27 patients (34.7%) in the neoadjuvant group (p = 0.08). Forty patients (8.1%) were classified as TP, 25 (5.1%) as ER/PR – HER2 +, 364 (73.2%) as ER/PR+ HER2 – and 68 (13.6%) as TN. Triple positive BC patients were more likely to have axillary SLN metastasis compared to other molecular subtypes (p < 0.006). Multivariate logistic regression revealed age (≤ 50 years) and TP tumors to be independent predictors of SLN metastasis. Odds ratio (OR) for TP vs other molecular subtypes was 3.646 (IC 95% 1.566-8.488). OR for age ≤ 50 years was 2.353 (IC 95% 1.554-3.559).

Discussion:

Conclusion: Her2 overexpression favours SLN metastasis in ER/PR positive tumors over other ER/PR status. Even with increased SLN metastasis, TP breast cancer patients do better than TN and ER/PR-HER2 positive patients. Molecular subtype classification may hold more potential value in the decision-making process in patients with BC than the currently staging system.

P3-07-22

Combined Approach for Staging the Axilla in Breast Cancer Patients with Clinically Negative Nodes Versus Sentinel Node Biopsy Alone.

Patel RK, Krol VY, Cibull ML, McGrath PC, Fjällskog M-L, Pirruccello EA, Szabunio AL, Samayoa LM. University of Kentucky, Lexington, KY; Uppsala University, Uppsala, Sweden; VAMC Lexington, KY

Background: Prognostic information and local control for managing the majority of clinically node negative (cN0) breast cancer patients may be achieved by sentinel node biopsy (SNB) alone and/or limited axillary dissections (LAD). Currently, 20-30% of clinically node-negative (cN-) patients have unnecessary surgery. This study compares the results from staging the axilla using SNB alone versus using the combined approach shown below.

Methods: Clinically node-negative (cN-) patients (n=176) were subclassified according to their primary tumor histology, axillary ultrasound (US) data, and US-guided fine needle aspiration (US-FNA) results, as follows: Low Risk (LR) for axillary metastasis (n=62); High Risk (HR) with normal axillary US (n=17); HR with US suggestive minimal N1a disease (n=23); HR with US suggesting N1a disease (n=52); HR with US suggesting N2-3 disease (n=22). All patients with (+) SNB or (+) US-FNA had Axillary Lymph Node Dissections (ALND). The number of (+) Sentinel Nodes (SN), Non Sentinel Nodes (NSN) and (+) LNs after a (+) US-FNA from each patient category was correlated with corresponding preoperative data. HR patients were defined as having grade II tumors > 1.5 cm and grade III tumors > 1.0 cm. US abnormalities in the axilla were interpreted as follows: minimal N1a disease equivalent to cortical defects <5 mm in 1-3 LN; N1a disease, cortical defects >5 mm in 1-3 LN and N2-3 disease, complete nodal replacement in ≥ 1 LN. LAD refers to level I dissections (1-5 LN).

Results: Three subgroups of patients were identified: Group A, patients not requiring ALND (128/176 = 72%); Group B, patients...
requiring ALND bypassing SNB (22/176 = 13%) and Group C, patients requiring LAD (26/176 = 15%). Preoperatively these 3 groups were categorized as follows: Group A included patients at LR for axillary metastasis, HR patients with normal axillary US, HR patients with axillary US suggesting minimal N1a disease and HR patients with axillary US suggesting N1a disease with (-) US-FNA; Group B included HR patients with axillary US suggesting N2-3 disease and (+) US-FNA; Group C included HR patients with axillary US suggesting N1a disease and (+) US-FNA. The post ALND characteristics for these 3 groups are summarized as follows: all Group A patients had N1a disease represented by ≤ 2 (+) LN, 94% (17/18) were SN (+) only, 85% (15/18) with 1 (+) LN and 15% with 2 (+) LN; in Group B, 20 patients had N2-3 disease and 2 patients had N1a disease, all Group B patients had > 2 (+) LN; in group C, 20 patients had N1a disease and 2 patients had N2 disease, and 77% had single (+) node disease.

Conclusion: By following this approach a more patient oriented method for staging the axilla can be implemented as follows: 1. SNB alone for LR patients and for HR patients with axillary US findings suggesting no axillary disease, minimal N1a disease and/or N1a disease with (-) US-FNA; 2. ALND for HR patients with axillary findings suggesting N2-3 disease and a (+) US-FNA; 3. LAD for HR patients with US findings suggesting N1a disease and (+) US-FNA. This approach would result in a 38% (48/176) reduction in the number of SNB and a 30% (22/66) reduction in the number of ALND. This translates in to $200,000 (30 - 40%) in procedure-associated savings.

P3-07-23
Intraoperative Molecular Analysis of Sentinel Lymph Nodes in Breast Cancer Using One Step Nucleic Acid Amplification (OSNA).
Chaudhry A, Massey E, Jenkins M, Calder C, Winters ZE, Rayter Z. Bristol Royal Infirmary, Bristol

Introduction: The OSNA method for the intra-operative analysis of sentinel lymph nodes (SLNs) in breast cancer has been introduced in 3 UK centres since 2007. The methodology uses a polymerase chain reaction to quantitate CK19, a cytokeratin specific to breast duct epithelial cells. OSNA provides “real-time” results on SLNs analysed as negative (-) or positive with either micro (+) or macrometastases (+). Methods: This is a single-centre prospective pilot study of all patients undergoing breast cancer surgery including sentinel node biopsy from February 2010 to May 2011. SLN identification was performed using a dual localization technique with peri-areolar Patente V blue dye and Te99 radio-active isotope. SLNs were cut into 4 slices labeled as A, B, C and D, respectively after the removal of all perinodal fat. In all SLNs, slices A and C were processed in OSNA and slices B and D underwent histological assessment by H&E staining. Slices A and C were prepared for OSNA analysis with the required reagent (Lynorhag), followed by centrifugation and homogenization. Micro-pipetted samples were processed against control specimens, to produce normalized and quantitative curve results. The primary outcome measure was to correlate the results of OSNA with histopathology for each SLN. A negative SLN resulted in no further axillary surgery compared to a level II dissection following the presence of micrometastases and a level II dissection following the detection of macrometastases. The total duration of OSNA for all SLNs was recorded in relation to delays in the completion of the intended breast surgery in each patient.

Results: 251 SLNs were analysed in 112 patients (mean age of 55 years). Comparisons between OSNA and histopathology were made in 116 nodes (34 SLNs analysed by OSNA only). SLN positivity was evident in 30 nodes (26%) comprising either macrometastases (n=13) or micrometastases (n=17). The OSNA sensitivity and specificity of was 93% and 89%, respectively. Accounting for a tissue allocation bias in the presence of micrometastases only, the specificity rose to 94% if these cases were excluded in the analysis. There was no correlation between SLN positivity and tumour grade, size or receptor status. The time to OSNA results were analysed in 75 patients undergoing 45 wide local excisions (WLE), of which 18 were wire localized; 23 mastectomies and 22 SLNB alone. The mean time for OSNA was 40.5, 51.8, 54 and 61.5 minutes for 1,2,3, and 4 sentinel lymph nodes respectively. Operation time was prolonged by a median of 20 minutes (range -48 to +65 minutes) WLEs were delayed by the greatest time

Exclusions: Nodes that were not available for histological comparison i) nodes weighing <0.05g (n=34) were processed whole. ii) Departmental agreement from mid March 2011 to process nodes whole via OSNA (n=51); 6 had micro or macrometastases.

Conclusion: OSNA prevented staged axillary surgery in 24 (21%) of patients. A median time of 20 minutes for the OSNA procedure is comparable with acceptable operating times. Current experience supports the use of OSNA for each individual whole SLN analysis. The pilot data has resulted in a prescribed change in policy to analyse the whole SLN using this technique.

P3-07-24
Accuracy and Cost Effectiveness of Frozen Section Examination of the Sentinel Lymph Node (SLN) in Ductal Carcinoma In Situ (DCIS) of Breast.
Ballehaninna UK, Santoro E, Schaefer SS, Blackwood MM, Chamberlain RS. Saint Barnabas Medical Center, Livingston, NJ; Maimonides Medical Center, Brooklyn, NY; Saint George’s University School of Medicine, West Indies, Grenada; University of Medicine and Dentistry of New Jersey, Newark, NJ

Background: SLN biopsy (SLNB) can avoid reoperation for axillary staging in DCIS patients upstaged to invasive breast cancer (IBC) following resection. Intraoperative frozen section (IFS) examination of the SLN is a widely accepted tool for IBC patients in order to select candidates for axillary lymph node dissection (ALND), and avoid reoperation. Until now, utility of IFS in DCIS patients has not been addressed.

Methods: Data from DCIS patients undergoing resection with SLNB (2000-11) was analyzed to determine the utility and cost of IFS exam and its impact on axillary management. A binomial regression analysis was performed to assess factors predictive of IFS positivity and SLN metastases (SLNM).

Results: 401 patients (core biopsy, N=276, or excisional biopsy, N=125) underwent partial (N=84) or total mastectomy (N=317) with SLNB. 77 patients (19.2%) were upstaged to IBC. SLNM was identified in 24 patients (5.9%). Immunohistochemistry (IHC) positive individual tumor cells (ITCs) was the most common pattern of SLNM (N=12, 50%) and macrometastases in 3 patients (12.5%). 365s
Conclusions: Most common patterns of SLNM in DCIS (ITCs/SLNmi) were identified only on routine pathologic assessment. Utility of IFS examination in DCIS patients is limited by poor sensitivity, high FNR and increased costs. Higher axillary nodal metastases are applicable to IFS positive patients (N=5). 11 of 24 patients with SLNN mand not change after biopsy and PST. The recently published Z11 trial of observation vs. axillary dissection for patients with a positive sentinel lymph node showed amazingly low axillary recurrence rates in both groups, and has the potential to dramatically alter practice. However questions remain about whether the Z11 population is representative of the larger population of breast cancer patients.

Methods

An institutional database was queried to identify all patients who underwent sentinel node biopsy at our breast center from 2004 to 2010. These cases were retrospectively reviewed to determine the percentage of patients who would have met the Z11 eligibility requirements, and the demographics and outcomes for this subset were compared to those for Z11.

Results

Out of 1215 patients undergoing sentinel node biopsy, 282 (23%) had at least one positive sentinel node. However when patients were eliminated who had T3 or T4 tumors, more than 2 positive sentinel nodes, neo-adjuvant chemotherapy, or who underwent mastectomy, only 88 remained who would have been eligible for Z11 (31% of those with a positive sentinel node). These patients are compared to the Z11 patients in the following table.

Comparison of Z11 trial and our database for T1 - T2 patients with 1 or 2 positive SN's and no neo-adjuvant chemotherapy.

<table>
<thead>
<tr>
<th></th>
<th>Z11 (n=89)</th>
<th>Our lumpectomy (n=88)</th>
<th>Our mastectomy (n=86)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median pt. age</td>
<td>55</td>
<td>57</td>
<td>53</td>
</tr>
<tr>
<td>Mean tumor size (cm)</td>
<td>1.7</td>
<td>1.9</td>
<td>2.4</td>
</tr>
<tr>
<td>% T1</td>
<td>31%</td>
<td>26%</td>
<td>22%</td>
</tr>
<tr>
<td>% micrometastases</td>
<td>41</td>
<td>17</td>
<td>22</td>
</tr>
<tr>
<td>% pos non-in*</td>
<td>27</td>
<td>30</td>
<td>45</td>
</tr>
<tr>
<td>Total pos nodes*</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>1-2</td>
<td>58%</td>
<td>61%</td>
<td>48%</td>
</tr>
<tr>
<td>3-4</td>
<td>22%</td>
<td>18%</td>
<td>29%</td>
</tr>
<tr>
<td>5 or more</td>
<td>14%</td>
<td>20%</td>
<td>23%</td>
</tr>
</tbody>
</table>

* for axillary dissection group in Z11

Our lumpectomy patients were generally quite similar to those of Z11 except ours had a lower percentage of micrometastases and a higher percentage of 4 or more positive nodes. As with most other published prediction models, the chance of having 4 or more positive nodes

P3-07-25

Sentinel Lymph Node Mapping in Breast Cancer after Primary Chemotherapy.

Bonardi S, Andreis D, Allevi G, Agugnini S, Milani M, Generali D, Bersiga A, Brizi MP, Dogliotti L, Berruti A, Bottini A. Azienda Ospedaliera Istituti Ospitalieri, Cremona, Italy; Azienda Ospedaliera Universitaria San Luigi di Orbassano, Università di Torino, Orbassano, Torino, Italy

Background: The high false negativity rate and the possibility of interference of primary systemic treatment (PST) and biopsy on the lymphatic drainage in the breast and axilla are the disadvantages of sentinel lymph node biopsy (SLNB) after PST. The primary aim of our study was to evaluate success rate for identification and isolation of sentinel lymph node (SLN) in patients treated with PST. Secondary aims were to verify if chemotheraphy and biopsy can really alter the lymphatic drainage, and to identify biological and clinical factors that can influence the accuracy of this technique.

Methods: Between June 2000 and April 2007, 176 consecutive operable or locally advanced breast cancer patients (T1-4N0-1M0) treated with antracyclin-based PST were enrolled in this single Institution study. Before performing a surgical biopsy and starting the treatment a lymphatic mapping was performed and the skin projection of the SLN location was then marked with permanent ink, with the aim to verify if the SLN marked did not change after biopsy and PST.

Results: The SLN was removed in 164 patients, with an identification rate of 93.2% (95% confidence interval (CI) = 89.4-96.9%). Fifty patients (30.5%) had metastatic involvement at SNB, and in 21 (42.0%) of them the SLN was the only positive node. Nine patients (5.5%) had a false negative SLN. The false-negative rate was 15.3% (95% CI = 7.1-32.9%). The SNB revealed a sensitivity of 84.7% (95% CI = 73.0-96.4%), an accuracy of 94.5% (95% CI = 90.1-99.0%) and a negative predictive value of 92.1% (95% CI = 85.8-98.4%). In 163 patients (99.4%) the SLN marked at baseline was the same removed at the end of treatment, while only in 1 case (0.6%) a different SLN was identified by the lymphatic mapping performed with radioactive colloid. According to clinical and tumor characteristics the rate of identification and removal of SNB was higher in patients aged <50 (95.6%) vs >50 (91.6%), with clinical node negative (95.1%) vs positive (88.6%) and with lower grade G2 (98.0%) vs G3 (91.2%). False negative rate of SNB was higher in patients aged >50 (17.9%) vs <50 (10.0%) and with clinical node negative (17.1%) vs positive (12.5%). Lymph node involvement was significantly associated with baseline ER positivity (p=0.0059 Chi-square test).

Conclusion: The identification rate, sensitivity and accuracy do not differ from other studies of SNB after PST. The false-negative rate is still high and we are performing analysis to identify biological and clinical features that can influence the accuracy of this technique. To our knowledge this is the first study with an in vivo demonstration that chemotherapy and biopsy do not alter the lymphatic drainage of the breast. We are performing exploratory analyses to evaluate the influence of false-negative rate of SNB on overall survival (OS) and progression free survival (PFS).
in our patients was directly proportional to tumor size and number of positive sentinel nodes, and inversely proportional to number of negative sentinel nodes.

Conclusions
A relatively small percentage of node positive patients at our institution actually met the eligibility requirements for Z11, but this subset was similar in most respects to the Z11 population. We have developed an algorithm that can be prospectively tested that will allow the majority of these patients to forego axillary dissection but that will exclude patients most likely to have 4 or more positive nodes.

P3-07-27
ROCK II Expression Can Be a Potential Marker of Non-Sentinel Lymph Node Metastasis in Breast Cancer Patients with Sentinel Lymph Node Involvement.

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(Background) It becomes controversy whether axillary lymph node dissection is mandatory performed for breast cancer patients with positive sentinel lymph nodes (SNs), given that more than half of patients with a positive sentinel lymph biopsy have no metastasis in non-sentinel lymph nodes (non-SNs). Rho-associated kinase (ROCK) is essential part in tumor invasion and metastasis, and its activation is reported to be potential marker of lymph node metastasis.

(Purpose) The purpose of this study was to evaluate the expression of ROCK II as predictive factor of non-SNs metastasis in breast cancer patients with positive SNs.

(Patients and Method) ROCK II protein expression was determined using immunohistochemical analysis on formalin-fixed and paraffin-embedded primary tumor samples composed of 119 SN-positive patients who underwent axillary lymph node dissection in National Kyushu Cancer Center. ROCK II expression was defined positive when there was strong intensity of cytoplasm staining.

(Results) ROCK II expression tended to be strong in invasive area, but weak in intraductal component. Of the 119 patients, 35 (29%) were determined to be positive for ROCK II expression. Patients with ROCK II positive tumor had significantly higher probability of non-SNs metastasis compared with patients with ROCK II negative tumor (20/35, 57% for positive; 28/84, 33% for negative, p=0.02). In multivariate analysis, positive ROCK II expression was significantly associated with non-SNs metastasis even after accounting for other predictive factors including tumor size, lymphovascular invasion, number of SNs metastasis and extra-capsule invasion (positive vs. negative, HR 2.6, p=0.04).

(Conclusion) These findings suggest that ROCK II expression can be a predictive factor for non-SNs involvement in breast cancer patients with SNs metastasis.

P3-07-28
One Step Nucleic Acid Amplification (OSNA) for Intraoperative Molecular Detection of Lymph Node Metastases and Micro-Metastases in Breast Cancer.

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Introduction: Ideally, intraoperative sentinel lymph node analysis in breast cancer should be automated, concordant with histopathology and practically applicable. One step nucleic acid amplification (OSNA), a highly sensitive intraoperative assay of cytotheratin 19 mRNA, is used for the detection of sentinel lymph node (SLN) macro- and micro-metastases in breast cancer. Guildford adopted the intraoperative OSNA “live” in December 2008 after undertaking a multicentre evaluation of its accuracy and high concordance with histopathology and here we present our two year data since its introduction.

Methods: Data was collected prospectively from 2008-10. All patients eligible for sentinel node biopsy were offered OSNA and operations were performed by five consultant breast surgeons. On detection of micro-metastases (+) and positive but inhibited metastases (i+), a level 1 axillary nodal clearance (ANC) and for a macro-metastasis (++), a level 3 ANC was performed.

Results: 471 patients had 999 SLN analysed, median age being 61. All except one were females. 72% (n=340) had wide local excision, 26% (n=120) underwent mastectomy and 2% (8) SNB alone. Mean tumour size was 18.3 mms. 80% (n=371) of the cases were IDC and 55% (n=256) had grade II tumour. 34% (n=161) had grade II tumour. 34% (n=161/471) had positive SLN who underwent axillary surgery at the same operation. This technique eliminates the need for a second operation in sentinel lymph node
positive patients and avoids the anxious wait for results in all, streamlining the patient’s cancer journey. OSNA upstages patients with micro-metastases and long term studies are needed to determine the clinical relevance of molecular micro-metastatic disease.

**P3-07-29**

*Validating the Lymph Node Ratio as a Prognostic Indicator among South East Asian Breast Cancer Patients.*

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Background: Several studies in Caucasian settings have suggested that the lymph node ratio (LNR, i.e. the ratio of the number of positive nodes to the total number of nodes excised) is a superior prognostic indicator for breast cancer as compared to the absolute number of nodes involved (pN stage). This study validates the prognostic performance of LNR in the South East Asian setting.

Methods: All patients diagnosed with non-metastatic invasive breast cancer at the National University Hospital (Singapore) or University of Malaya Medical Center (Kuala Lumpur) between 1990 and 2007 and with information on axillary nodes removed and involved were included for analysis (n = 1589). Multivariate Cox regression analysis was performed to evaluate the prognostic value of the LNR [low (<0.2), intermediate (0.2 to 0.65) and high risk (>0.65 to 1)] and pN staging [pN1, pN2 and pN3] for all cause mortality. Patients staged pN0/LNR 0 were excluded from analysis.

Results: According to the LNR classification, 758 patients were categorized as low risk, 574 as intermediate risk and 257 as high risk LNR. For classic pN staging, 879 were pN1, 447 pN2 and 263 pN3. Women in the intermediate risk category (0.2 to 0.65) had a 1.5 fold increased risk of death [Adjusted hazard ratio (HRadj) of 1.5 (95%CI, 1.2 to 1.9)], and women with high risk (>0.65 to 1) LNR had a 3.2 fold increased risk of death [HRadj of 3.2 (95%CI, 2.6 to 4.1)] as compared to the low risk women (>0 and <0.2). Similarly, women with pN2 disease had a 1.9 fold increased risk of death [HRadj of 1.9 (95%CI, 1.5 to 2.4)], and women with pN3 disease had 3.1 fold increased risk of death [HRadj 3.1 (95%CI, 2.5 to 3.9)] compared to pN1 patients.

Conclusion: Among South East Asian breast cancer patients, both the Lymph Node Ratio and the pN Staging system seem to be equally good at predicting all cause mortality.

**P3-07-30**

*Applying the Findings of the Z11 Trial to a UK Practice.*

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Introduction: The finding of a positive sentinel node is currently managed by further Level III axillary lymph node clearance (ALNC). The rationale behind this approach is that of local disease control, but there is little evidence that axillary lymph node clearance results in a reduction in axillary recurrence or in mortality. ALNC is however associated with increased morbidity (lymphoedema, nerve damage, reduced shoulder function) and significantly prolongs hospital stay. The Z11 trial suggests that ALNC following positive sentinel node biopsy does not result in lower axillary recurrence rates compared to the group in whom clearance was not undertaken and has obvious implications for evidence-based practice. The Z11 trial has strict inclusion criteria (T1/T2 tumour, breast conservation surgery) with all patients receiving adjuvant whole breast radiotherapy and systemic chemotherapy or endocrine therapy. For patients who do not meet the patient population of Z11, such as women undergoing mastectomy, the Sloane-Kettering predictive normogram provides an estimate of risk for residual axillary disease after positive sentinel node biopsy and the estimate of this risk may inform the clinical decision to clear the axilla based on individual cancer characteristics.

Methods: Our population comprises both symptomatic and screening patients, with an axillary positivity rate of approximately a third. This study was undertaken to assess the impact that Z11 would have on our practice. We prospectively maintained records were searched for ALNC patients treated between 2003 and 2011. We assessed the number of node-positive patients conforming to Z11 criteria and the number who demonstrated residual axillary positivity at clearance after a positive sentinel node. The axillary recurrence rate for this group after ALNC was recorded. We calculated the number of clearances that could have been avoided, and extrapolated the reduction in morbidity in lymphoedema and nerve damage using the audited incidence of these complications in our institution. We calculated the financial cost saving in terms of theatre usage and hospital stay. In addition, we assessed whether the Memorial Sloane Kettering predictive normogram is useful in the prediction of residual axillary disease for the group of patients excluded from the Z11 cohort.

Results: 1601 patients underwent axillary staging. 65% of our patients with node-positive disease were identified pre-operatively with ultrasound and biopsy and proceeded directly to ALNC. Our overall axillary recurrence rate was low (<1% at 5 years). 26% of our patients would not meet the criteria for Z11, predominantly due to the requirement for mastectomy. Of those that met the Z11 criteria, 60% had no further axillary disease at clearance and a further 25% demonstrated low volume (1-3 positive nodes) residual disease only. The Memorial Sloane Kettering normogram can be used in estimating risk of residual axillary disease in patients undergoing mastectomy.

Discussion: Using these criteria nearly 25% of axillary clearances in our population of breast cancer patients could be avoided with obvious cost savings both in terms of morbidity and finance, considerations that are important in planning our service for the future.

**P3-07-31**

*Intra-Operative Assessment of Sentinel Lymph Nodes in Breast Cancer with Touch Imprint Cytology (TIC) in 460 Consecutive Patients.*

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Background: Sentinel node biopsy (SNB) accurately predicts the axillary lymph node status in patients with breast cancer. The sentinel lymph node (SLN) is evaluated by routine histology, requiring 3-5 days for the results and a second operation if the SLN is positive. However a second operation can be avoided if a reliable intra-operative assessment of the SLN was available. The techniques used for intra-operative assessment of the SLN include frozen section, touch imprint cytology (TIC) and more recently molecular biology assays. The aim of this study was to evaluate the accuracy of TIC in the assessment of the SLN.

Methods: A prospective study to include 460 patients with breast cancer who underwent SNB and had the sentinel node assessed intra-operatively with TIC. The SLN was bisected and a touch imprint was made on to a slide. The imprint was stained with
Giemsa stain. Permanent sections were evaluated with H&E and immunohistochemical staining. The TIC results were compared with the final histology of the SLN.

**Results:** 766 SLN’s were harvested from 460 patients (Mean - 1.66). Of the 460 patients, TIC was falsely negative in 50 (24%) patients. No patients had false positive results. Negative predictive value was 87%. The accuracy rate was 89%. 94 patients were positive on histology. TIC was positive in 44 patients and negative in 50. In the sensitivity, specificity and positive predictive value of TIC was 47%, 100% and 100% respectively. By acting on the results of TIC, 44 patients (47%) had an axillary clearance at the primary operation and were thus spared a second operation.

**Conclusion:** TIC in our cohort of 460 patients and 766 sentinel lymph nodes had an accuracy rate of 89% and specificity of 100%. Our study compares favourably with published literature, confirming that TIC is a simple, quick, reliable and reproducible technique that can be used for intra operative assessment of the SLN. A sensitivity of 47% means that about half of the SLN positive patients were spared a second operation. We had hoped that with more experience, our sensitivity would improve but since this has not been the case we are now moving towards molecular biology assays (OSNA) to assess the sensitivity would improve but since this has not been the case we are now moving towards molecular biology assays (OSNA) to assess. TIC is a simple, quick, reliable and reproducible technique that can be used for intra operative assessment of the SLN. A sensitivity of 47% means that about half of the SLN positive patients were spared a second operation. We had hoped that with more experience, our sensitivity would improve but since this has not been the case we are now moving towards molecular biology assays (OSNA) to assess.

**P3-07-32**

The Role of Axillary Ultrasound in the Detection of Metastases from Primary Breast Cancers.

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**Background:** The value of ultrasound (US) in the preoperative evaluation of axillary nodes has yet to be completely clarified. Preliminary experience with this technique in our institution was examined.

**Methods:** Patients with a radiographic or palpable abnormality of the breast had simultaneous breast and axillary US. The exams were performed by dedicated breast radiologists using a 12 MHz linear array transducer (HDI 5000: Philips Ultrasound). Results were reviewed for all patients with invasive cancers who were cN0 and had definitive surgical procedures between June 2006 and May 2008. Criteria for abnormal lymph nodes were loss of reniform shape, focal or diffuse cortical thickening, or eccentric/replaced fatty hilum. US-guided biopsies were done using a 16g spring-loaded core biopsy device (16g MD TECH SuperCore). Patients with positive axillary node biopsies bypassed sentinel lymph node biopsy (SLNB) and had axillary dissection, whereas those with sonographically normal nodes or benign/non-diagnostic biopsy results had SLNB at the time of definitive surgery.

**Results:** Of 128 patients diagnosed with invasive cancer, 23 (18%) had abnormal axillary US at the time of initial diagnosis. Biopsies were performed in 18 of the 23, of which 12 (67%) were malignant and 6 (33%) were benign. Ultrasounds were negative in 105 (82%) patients. SLNB was done in 110 patients: 103 with negative US; 4 patients with abnormal US but negative axillary biopsies; 2 patients with abnormal US but no core biopsies; and 1 patient with a positive US biopsy. SLNB was negative in 91 (83%) patients and positive in 19 (17%). The node-positive status was N1a in 14 patients and N1mic in 5. Axillary dissection was done in 32 (25%) of 128 patients, comprised of 11 patients with US-guided positive biopsies, 12 with positive sentinel nodes, 2 with US-guided negative biopsies, 2 with negative ultrasounds, 3 with unbiopsied abnormal ultrasounds, and 2 with a false-negative SLNB. For determining axillary metastases with US, sensitivity was 16/31 (52%), specificity was 90/97 (93%), positive predictive value was 16/23 (69%), and negative predictive value was 90/105 (86%).

**Conclusions:** US examination was a valuable method of evaluating the axilla in newly diagnosed breast cancers. Of 32 patients having an axillary dissection, abnormal US eliminated the need for SLNB in 17 (53%). Patients with US-guided positive nodes were submitted to axillary dissection without SLNB. Therefore, we were unable to determine how often US identified an abnormal non-sentinel node, thus upstaging the axilla relative to SLNB alone. This question should be the topic of further clinical study.

**P3-07-33**

Are Single Node Metastases More Common in Patients with Breast Cancer in the Sentinel Node Era?

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**Background:** The axillary recurrence rate after axillary lymph node dissection has been reported as approximately 3%. Since the introduction of sentinel node biopsy (SNB), it has become apparent that, there is a significant decrease in axillary recurrence, ranging from 0.26% to 0.9% in published literature. One of the concerns that arises from this data is that, the sentinel node may have been missed when routine axillary node clearance were being performed. The aim of this study was to compare the results of a cohort of patients who had an axillary staging procedure prior to the introduction of SNB (axillary clearance) with patients after the introduction of SNB in relation to the number who had only one positive node in the axilla.

**Methods:** From June 2003 to November 2010 data was collected on demographics, histopathology and procedure performed. All patients with invasive breast cancer prior to April 2005 had an axillary node clearance (ANC) with either wide local excision or mastectomy. Since April 2005 all patients with invasive breast cancer had a SNB and patients with a positive sentinel node (SN) underwent ANC. Patients with a negative SNB had no further axillary surgery.

**Results:** 702 patients had axillary node surgery between June 2003 and November 2010. 251 patients had axillary node clearance (pre SN period) and 451 had SNB (SN period). Average age was 57 years. 509 patients (156 in pre SN period and 353 in post SN period) had no nodal metastases. Amongst the 193 node positive patients, 100 had single node involvement. In the pre SN period 33 (34.7%) patients had single node involvement, compared to 67 (68.3%) in SN period. The histopathological type of cancer did not vary significantly between the one node positive, multiple node positive and node negative groups.

**Conclusion:** Our study confirms a significant rise in patients with a single node positive in the sentinel node period. This could be due to the SNL being “missed” during surgery when an axillary node clearance was performed, which would explain the reported higher axillary recurrence rates in the pre sentinel node period compared to the sentinel node period. Another possibility is that the SN is undergoing more detailed histopathological assessment as there are fewer number of lymph nodes to be examined.
P3-07-34
Occult Metastasis in Sentinel Node: Should This Affect the Clinical Decision Making? A Systematic Review and Meta-Analysis.

Background: The role of occult metastases (micrometastasis or isolated tumor cells [ITC]) in sentinel lymph nodes (SLN) remains a field of debate and speculation. The purpose of this systematic review and meta-analysis was to investigate the prognostic relevance of occult metastases in SLN.

Methods: We searched PubMed, without year or language restriction through June 2011, for studies on patients with invasive breast cancer with micrometastasis or ITC in the SLN and presented Hazard Ratios (HR) on Overall Survival (OS) / Disease-Free Survival (DFS) or enough data for HR-calculation. We used fixed- or random-effects meta-analyses, as appropriate, to calculate pooled estimates of HR.

Results: Fifteen studies were considered eligible. Of those, 3 studies were excluded due to the lack of data for HR-calculation. Both the presence of ITC (5 studies; 1264 patients with ITC) and micrometastasis (10 studies; 1093 patients with micrometastasis) were associated with worse DFS (pooled HR 1.29, 95% Confidence Interval [CI]: 1.09-1.52, P-value = 0.002 for ITC, pooled HR 1.45, 95% CI: 1.28-1.64, P-value < 0.001 for micrometastasis). No significant differences were observed regarding OS neither for the presence of ITC (5 studies; 877 patients with ITC; pooled HR 1.20, 95% CI 0.98-1.46) nor for micrometastasis (8 studies; 1094 patients with micrometastasis; pooled HR 1.36, 95% CI: 0.98-1.88).

Conclusion: Based on the current evidence, it seems that the presence of occult metastasis in SLN is of prognostic significance in regard to DFS. However, the clinical significance of this difference is questioned since it cannot be translated into differences in OS. In anticipation of randomized trials aimed at evaluating the optimal management of each category of tumor burden within SLN, current evidence suggest a limited clinical significance of occult metastases in SLN.

P3-07-35
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Purpose: Axillary lymph node dissection (ALND) is recommended for breast cancer patients with sentinel lymph node (SLN) metastasis, but further nodal disease is not always present. Several models exist for predicting non-sentinel lymph node (non-SLN) metastasis in SLN metastasis. This study evaluated and compared the predictive values of the Memorial Sloan-Kettering Cancer Center (MSKCC) nomogram and the Stanford nomogram, which were conveniently available online, and clarified their usefulness for the micrometastasis or isolated tumor cell (ITC) subgroup.

Methods: Data from 89 patients with positive SLN biopsy who underwent ALND were used, including 59 patients with micrometastasis and 30 with micrometastasis/ITC. The predicted probability of non-SLN metastasis was calculated using a computerized model from the websites for each nomogram. Results were compared using area under the curve (AUC) of the receiver operating characteristics curve for each model. False-negative and false-positive rates were also calculated.

Results: AUC for the entire population was 0.701 with the MSKCC nomogram and 0.756 with the Stanford nomogram. AUCs of micrometastasis and micrometastasis/ITC were 0.680 and 0.469 with the MSKCC nomogram and 0.676 and 0.574 with the Stanford nomogram, respectively.

Although false-negative cases were not identified, false-positive rates were high in both subgroups with these nomograms.

Conclusions: This independent comparison found no significant difference between the two nomograms. In our results, these nomograms could not reliably predict positive non-SLN in cases with SLN micrometastasis/ITC. Further validation in other patient populations is needed.

P3-07-36

Background: The Memorial Sloan-Kettering Cancer Center (MSKCC) developed a nomogram to predict the presence of sentinel lymph node (SLN) metastasis in breast cancer patients. In our study, The MSKCC nomogram performance for prediction of SLN metastases was assessed in Chinese breast cancer population. A new model (Shanghai Cancer Center Nomogram, SCC nomogram ) was developed with clinically relevant variables and possible advantages.

Methods: Data were collected from 771 patients with successful SLN biopsy who were treated during March 2005 to June 2010. Touch imprint cytology (TIC) and serial section with H&E staining were performed routinely on each sentinel node. 580 SLN biopsy procedures from March 2005 to November 2009 were used as training group to validate the MSKCC nomogram and assessed with multivariable logistic regression to predict the presence of SLN metastasis in breast cancer. The predictive accuracy of MSKCC nomogram was assessed by calculating the area under the receiver-operating characteristic (ROC) curve (AUC). The SCC nomogram was created from the logistic regression model. The new model was subsequently applied to 191 sequential SLN biopsies from January 2010 to June 2010 as the validation group.

Results: It was shown that age, tumor size, tumor type, histological grade, lymphovascular invasion and neural invasion was correlated with the probability of SLN metastasis by univariate analysis (P<0.05). By multivariate analysis, tumor size, histological grade and
lymphovascular invasion were identified as independent predictors of SLN metastasis. The SCC nomogram was then developed with four variables associated with SLN metastasis: age, tumor size, histological grade and lymphovascular invasion. The new model was accurate and discriminating, with AUC of 0.773 when applied to the validation group, as compared to the MSKCC nomogram with AUC of 0.754 in the modeling group. The trend of actual probability in various decile groups was comparable to the predicted probability. For predicted probability cut-off points of 7% and 15%, the false-negative rates of SCC nomogram were 0% and 8.1%.

Conclusion: As far as we know, this is the first study designed to evaluate the MSKCC nomogram and develop a new nomogram in Chinese early breast cancer population. Compared to the MSKCC nomogram, the SCC nomogram was developed with similar AUC but less variables and lower false-negative rates for low-probability subgroups. It could provide a more acceptable clinical accessory in the preoperative discussion with patients, especially in the very low risk of patients. For those patients, the SCC nomogram could be used to safely avoid a SLN procedure, thereby reducing postoperative morbidity, whereas the rate could be as high as 7% in the literature. Although the SCC nomogram that predicts metastasis of breast cancer in the sentinel lymph node performed well in Chinese breast cancer population, it is imperfect. The SCC nomogram was developed and validated in the single instite. The SCC model should be validated in different patient groups before it is demonstrated to be reproducible and would be applied widely.


Hegg R, Mattar A, Gebrim LH, Emenerciano K, Pinotti M, Perdicaris M, van Eyll B, Franke F, Pinczowski H, Freitas Jr R, Jendiroba D, Borges G, Queiroz G, Nascimento YV, Gampel O, Matias C, Buel V, Strepassos E, Delgado G, Perola Byington Hospital, Sao Paulo, SP, Brazil; Liga Norte Riograndense; EGIMAJAP; Instituto Santista de Oncologia; Servidor Publico do Estado de Sao Paulo; Nucleo de Oncologia da Bahia; Universidade Federal do Para; Hospital Sao Rafael; Hospital Santa Lucinda

Background: Breast cancer is the most common type of cancer among Brazilian women with almost 50,000 new cases per year. There are few data regarding the clinical presentation, treatment and specially outcome of this population. Brazilian health system is composed by Public institutions (Pu), Private centers (Pr) and some institutions that assist both Public and Private patients (PuPr).

Material and Methods: We collected data from 17 cancer centers distributed throughout Brazil among Pu; Pr and PuPr centers. We’ve analyzed data from 1-clinical characteristics, 2-pathologic characteristics and 3-type of treatment received among 2435 patients from May 2008 to May 2009.

Results: Mean age at diagnosis was 53 years, with about 30% below age 50. Most of the cases were Invasive Ductal Cancer (83%). Stage 0 was seen in 3.2%, Stage I in 21.8%, Stage II in 46.6%, Stage III in 24.6% and Stage IV in 3.9%. Clinical Stage III + IV was seen in 18.5% of the Pu institutions, only 3.7% of the Pr ones and about 6.2% among those PuPr. Hormone receptors were positive in 55%. Her-2 was overexpressed in 27.3% of the patients, and triple negative were seen in 11.6%. Most of the patients were submitted to surgery (92.9%). In Pu institutions only 36% of the patients were submitted to Breast Conserving Surgery (BCS) and in the Pr institutions 49.4% of the patients were submitted to BCS and in the PuPr 47%. Breast reconstruction was made in 15.8% and did not differ between Pu and Pr institutions. Sentinel node biopsy was done in 30.6% of the patients (26.8% of the patients from the Pr institutions and 26.8% of the Pu ones and 33% among PuPr). Neoadjuvant treatment was done in 21.5% of the patients (Pu=27.2%; Pr=13.9% and PuPr 13.2%). Most of this neoadjuvant treatment was chemotherapy (93.8%) and only 4.3% was hormone therapy (HT). 30% of the patients received AC, 41% A+taxane and 18.9% FAC/FEC. Besides we have almost 30% of Her-2 overexpressed only 1.1% of the patients received trastuzumab in the neoadjuvant setting. Tamoxifen was used in 48.3% when neoadjuvant HT was done, and aromatase inhibitor (AI) was used in 34.5%. Most of the patients received any kind of adjuvant treatment (89.2%). Chemotherapy was done in 76.6% and hormone therapy in 69.8%. When chemotherapy was used the preference regimen was FAC/FEC (27.3%), followed by CMF (17.5%) and AC (11.9%). Trastuzumab was used in only 5.8% of the patients (Pu=6.8%, Pr=18.3% and PuPr 3% among all patients that received chemotherapy). In the adjuvant setting, Tamoxifen (TAM) was prescribed in 69.8% of the cases (Pu=87.6%, Pr=79.6% and PuPr 78.8%), AI in 8.2% (Pu=5.9%, Pr=9.3% and PuPr 13.8%), and sequential TAM/AI in 6.6% (Pu=61%, Pr=8.3% and PuPr 6.4%). About 17% of the patients had metastasis.

Conclusions: There are important differences between the public and private institutions in Brazil, the patients from the Pu institutions were five times more likely to be diagnosed in stage III or IV, they usually receive neoadjuvant treatment, and when surgery was done, most of them were treated with radical procedures. Besides the overexpression of Her-2 (30%) a minority of the patients received treatment with trastuzumab even for the Private centers (high cost for a developing country).

P3-07-38 Selective Omission of Blue Dye in Patients Undergoing Sentinel Lymph Node Biopsy for Breast Cancer.

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Background: Combined blue dye and radioisotope colloid injection with scintigraphy is standard practice for mapping and biopsy of sentinel lymph node in breast cancer within the UK. Whilst this combination aids sentinel node detection rate, blue dye has a number of possible adverse effects including anaphylaxis (reported as 1-3% of patients), semi-permanent skin tattooing and staining of bodily fluids which may cause distress for the unwary patient. It can also cause obscuration of the operating field, making identification and dissection of planes more difficult.

Methods: Patients undergoing sentinel lymph node biopsy (SLNB) using either a combination of blue dye and radioactive colloid, or radioactive colloid alone during a 14 month period were compared for identification rate, node harvest number and final positive rate. A total of 122 axillary sentinel node biopsies in 121 patients were identified. All patients scheduled for sentinel lymph node biopsy had intradermal injection of radiocolloid and lymphoscintigram pre-operatively and were checked for radioactive intensity with gamma probe on the table before draping for surgery. Patients with good signal proceeded to surgery without blue dye. Those with more than 3 nodes (n=9), radioisotope skin contamination (n=2), absent signal on scintigraphy (n=7) and/or with weak pre-operative radioactive
signal (n=22) were given 2 millilitres of patent V dye subdermally in the periareolar region. Sentinel node biopsy then proceeded in the standard fashion. Data was also collected from the year prior to be used as a control group, where the combination of blue dye and radioisotope was used for all patients (n=90), and compared with the group receiving radioisotope alone.

**Results:** The rate of identification for single agent and dual agent was 100% and 97.5% respectively, with no significant difference in mean node harvest using radioisotope alone (1.80) as compared to combined technique (1.87 p= 0.88, 95%CI 0.39 to 0.34). There was no significant difference in the number of patients with positive nodes on final histology when using single agent (13 (14%)) when compared to the dual agent technique (10 (25%) p=0.21). There was also no difference in the rate or number of node harvest in the group who underwent the single agent technique when compared to the control group (100% identification, mean harvest 1.84, p=0.77, 95% CI 0.36 to 0.27), with no difference in node positivity (16 (17%) p=0.68). No intra operative adverse reaction was reported in any of the groups.

**Discussion:** This study has shown no significant difference in the localisation rate when selectively omitting blue dye in suitable patients compared to the combined technique. This may be due to significant operator experience or change in the method of radioisotope injection since initial studies were performed. In this study, 86 out of 126 SLNB proceeded without blue dye, meaning not only a reduction in the number of patients with minor adverse effects, but also a potential reduction in severe adverse reaction of 68% or 2 patients per year within this breast unit.

**P3-07-39**

**Where To Look for Sentinel Lymph Node in Breast Cancer.**

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**Hypothesis:**
The sentinel Lymph node (SLN) is not only the first extra mammary point of metastatic spread, it also undergoes reactive changes. Sonographically this is evident through an increased width of the hypo-echoic cortex of the lymph node. We examined, if it is possible to localize SLN on sono-morphological features, if it is sonographic detection comparable with the radio nuclide tracer method and if false negative rate (FNR) of SLNB can be reduced by ultrasound.

**Method:**
From 1/2001 to 5/2003, 117 patients with breast cancer where prospectively examined for SLN localization using sonography in addition to radio nuclide -Tc99m- and/or patent blue-methods. The lymph node identified as the SLN in ultrasound was marked using a hook-wire. The sonographic parameters used to identify the SLN were: The node with the widest hypo echoic cortex, located in the caudal axilla and the closest to the glandular tissue and with the greatest diameter.

**Results:**
In all 117 patients, in the region of the caudal axilla, an enlarged, reactive, either tumor free or metastatic SLN could be verified. The location of the SLN was in all cases on the lateral-caudal thoracic wall and consistent with the lymph node group at the lateral border of the pectoralis major. The sonographic detection rate of the SLN was 86%. 70.6% of the sentinel lymph nodes where affected by metastatic spread. In those patients with a pT1a-c-tumor the rate of SLN metastatic spread was 20%. The false negative rate (FNR) for SLN localization with Te99mBlau was 10.6 %. Experience has shown that the FNR increases with increasing degree of metastatic spread to the SLN. With the addition of sonography of the axilla the FNR could be decreased to 3.5%.

**Sonographic SLN detection rate: 86% (n=101/117)**

**False-negative-rate (Blue/Tc) 10.7% (n=6sln-/56N+)**

**False-negative-rate (Blue/Tc + Sonography) 3.5% (n=2sln-/56N+)**

**Conclusions:**
The false-negative-rate of detection of suspected lymph nodes can be reduced using sonographic localization of the SLN. Sonography of the axilla should therefore be an integral part of and standard in the sentinel node-biopsy of breast cancer patients.

**P3-07-40**

**Impact of the Sentinel Lymph Node Procedure on the Detection of Positive Lymph Nodes in Breast Cancer.**


**Introduction:**
The objective of the sentinel lymph node (SLN) procedure in breast cancer is to perform an accurate axillary staging and provide good local control, while sparing the patients the morbidity of an axillary lymph node dissection (ALND). Since its routine clinical use, questions have been raised concerning the upstaging of a subgroup of node-negative patients and an increase in the overall percentage of node-positive patients.

The goal of our study was to investigate the impact of the SLN procedure on the detection of positive lymph nodes.

**Patients and methods:**
We included 1119 consecutive breast cancer patients from one center (2007-2009) who underwent primary surgery for a breast cancer smaller than 5 cm diameter without clinical involvement of axillary lymph nodes. 31 patients had a bilateral breast cancer.

**Results:**
We compared the SLN biopsy group (n=90) with the ALND group (n=828). In univariable analysis, the rate of node-positive patients was not significantly different in the two groups (16% versus 17%, p=0.68). The difference in the rate or number of node harvest in the group who underwent SLN biopsy as compared to ALND was not significant (p=0.77). The false-negative rate (FNR) of SLNB can be reduced by sonography.

**Conclusion:**
For comparable tumors, the chance of detecting positive lymph nodes is not lower when applying SLN procedure compared to ALND. The results even point in the direction of a higher detection rate with SLN.
However, care should be taken in making this conclusion given a non-significant result.

<table>
<thead>
<tr>
<th>Table 1: Univariable and multivariable analyses</th>
<th>Univariable</th>
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**P3-07-42**

**Lymphovascular Invasion Best Correlates with Presence of Nodal Metastasis in Sentinel Lymph Node Biopsy.**

Ahsar MS, Adelekan MO, Patel E, Cotterill S. North Manchester General Hospital, Manchester, United Kingdom; University of Manchester, Manchester, United Kingdom

Background. Sentinel lymph node biopsy (SLNB) biopsy is routinely practised for axillary staging with 25-30% having positive SLN. Tumour size is the most important parameter taken into consideration in decision making in node negative patients with a size cut off for 4-5cm dependent on different units. Lymphovascular invasion (LVI) and Ki-67 a cell-cycle antigen are known important prognostic markers along with the tumour grade, oestrogen receptor and herceptin receptor status.

Aim. To examine whether lymphovascular invasion, Ki-67 or any other factors can be used as a predictor for axillary lymph node involvement and hence prognosis.

METHODS. A prospective study of 264 patients with invasive breast cancer undergoing SLN biopsy between January 2009 and December 2010. Histopathology reports were reviewed regarding LVI, Ki-67, grade, oestrogen, Progesterone and Herceptin receptor and SLN status. Stats direct was used to analyse data. Logistic regression was used and p-value calculated.

RESULTS. LVI (p value=0.0001) and size(p value=0.0273) were the two most significant factors associated with node positivity. Grade of tumour had a p-value of 0.0825 and Ki67 had a p-value of 0.5217 which were not significant.

**Factors affecting nodal status**

<table>
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**DISCUSSION.** LVI is the factor which best correlates with presence of metastasis in sentinel lymph node. If this information is available at the time of decision making, it should be strongly considered and pathologists should be encouraged to provide the information. In the absence of LVI, size still remains the best predictor of sentinel lymph node metastasis pre-operatively although consideration of other factors, such as the grade is definitely relevant.

**P3-07-43**

**The Impact of Timing in Sentinel Lymph Node Biopsy in Primary Breast Cancer.**

Gäth U, Schmid SM, Myrrick ME, Obermann EC, Viehl CT, Rochlitz C, Forrer F. University Hospital Basel, Basel, Switzerland

Background. There is minimal data concerning the impact of timing on the radiolabeled colloid marked sentinel lymph node (SLN) procedure. Some surgeons believe that the operation should be performed as the first case in the morning because a delay could theoretically lead to a variation in number of lymph nodes removed; there could be a) an artificially higher number due to diffusion of the radiolabeled colloid, or b) a lower number due to decay of the radiolabeled colloid. We examine the correlation between the number of SLN removed and the time of day when the SLN procedure was performed.

METHODS: Between January 2003 and December 2009, 391 SLN procedures were performed in histologically confirmed invasive breast
carcinomas at the University Women’s Hospital Basel, Switzerland. In line with the procedure defined by the “Swiss Multicenter Sentinel Lymph Node Study in Breast Cancer Patients”, each patient underwent standard labeling, removal and pathological diagnosis of the SLN. In all cases, lymphatic mapping was performed with 99mTc-labeled nanocolloid the day before the operation between 2.00 and 3.00 p.m. In addition, 2-4 ml of patent blue V was injected 5-10 minutes prior to incision. The radiolabeled colloid marked SLN were intraoperatively identified by the use of a handheld gamma probe. All samples were sent for immediate assessment to the local Institute of Pathology. The number of lymph nodes was determined and frozen sections were performed.

The timing of the SLN removal can be pinpointed unequivocally in all cases, since the pathology report records the arrival of the tissue samples for frozen section to the exact minute. In order to analyze the correlation between the timing of surgery on the operation day and the number of lymph nodes removed, we calculated the minutes from the beginning of the operating schedule in the morning (8.00 a.m.) until the arrival of the tissue samples in the frozen section laboratory (including subtraction of fifteen minutes handling and transport time). In order to calculate the association between the timing of the operation on the operation day and the number of the SLN removed, nonparametric spearman correlation (Spearman-Rho) was used; negative values of the coefficient rho indicate a descending trend.

Results: In 391 procedures, 928 SLN (median: 2; range: 1-11) were removed. Although blue dye was used in all cases, in the majority of cases (50.8%, n=471) the SLN were marked exclusively by radiolabeled colloid and only detected by the use of the handheld gamma probe. Only in 27 of the 928 SLN (2.9%), did the surgeon identify the lymph nodes through the blue coloration alone. The median time between start of the operation timetable and the SLN removal was 150 minutes (range: 25-420). There was a weak negative association between timing and the number of SLN removed (Rho=-0.070; p=0.168), i.e. there was a small, non-significant decrease in the number of SLN removed at a later time in the day.

Conclusion: After mapping SLN the previous day by using radiolabeled colloid method, the timing on the day of the operation has no relevant impact on the number of SLN removed.

P3-07-44
Noguchi M, Ohno Y, Nakano Y, Noguchi M, Kosaka T. Kanazawa Medical University, Kahoku, Ishikawa, Japan

Background: The axillary reverse mapping (ARM) procedure is based on the hypothesis that the lymphatic drainage from the upper arm is different from that of the breast. However, the oncologic safety of the procedure has not yet been determined.

Methods: The ARM nodes were identified using a fluorescence imaging system. Sentinel lymph node (SLN) biopsy was performed in patients with clinically uninvolved nodes. If the SLN was positive, ALND was performed with removal of ARM nodes. Otherwise, the identified ARM nodes were preserved unless they were the same as the SLN.

Results: The ARM node was identified in 30 of 91 patients who underwent SLN biopsy, and it was the same as the SLN in 23 patients. However, in 13 patients with a positive SLN who subsequently underwent ALND, ARM nodes were tumor-free when they were not the same as the positive SLN.

Conclusions: There are limits to the principle of non-overlap between breast and arm nodes. However, it may be feasible to spare ARM nodes during ALND in patients with clinically negative nodes.

P3-07-45
Role of SPECT-CT in Detecting Sentinel Lymph Nodes in Patients with Ipsilateral Breast Cancer Recurrence and Previous Axillary Lymph Node Dissection.
Cordoba O, Perez-Ceresuela F, Roca I, Mendoza C, Fortadellas T, Espinosa-Bravo M, Rodriguez J, Peg V, Rubio IT, Xercavins J. Hospital Universitari Vall d’Hebron, Barcelona, Spain; Universitat Autònoma de Barcelona, Barcelona, Spain

Background: Use of sentinel lymph node dissection in patients with ipsilateral breast cancer recurrence and a previous axillary lymph node dissection (ALND) is still controversial. Although previous reports have showed extra-axillary drainage in 40-60% of patients, the clinical significance of this drainage is unknown. SPECT-CT may help to localize aberrant sentinel nodes.

Material and Methods: Between 2008 and 2011, SLN were performed in 25 patients with ipsilateral breast cancer recurrence and previous ALND. The day before surgery 99Tc nanocolloid was injected retroareolar in the affected breast and injected intratumorally when the recurrence was after a mastectomy. Lymphoscintigraphy was obtained in all patients and a SPECT-CT was performed in all cases even when planar images showed no drainage. During surgery, the sentinel node was identified using a gamma probe. During the procedure the surgeon decided to remove the sentinel node if it was considered technically feasible. The project was IRB approved and all patients signed an informed consent.

Results: Records from the previous ALND showed 9 patients with positive axillary nodes with a mean of 19 (range 10-35) lymph nodes excised. Twenty four patients had undergone a lumpectomy and 1 patient a mastectomy. After the injection, the SPECT-CT showed at least one hot spot in 20 patients, with a mean of 1.8 hot spots (range 0-5). Hot spots on SPECT-CT were located as follows: in 10 patients axillary, 1 subclavicular, 7 internal mammary, 1 intramammary, 1 mediastinic and 7 patients had a contralateral axillary hot spot. In 4 patients we don’t found any hot spot on SPECT-CT. After excising 2 axillary nodes and 1 intrammary node in 3 patients, the pathologist didn’t identify any lymph node in the hot spot removed. This are considered as false positives (15%). In 4 patients although hot spots were identified, sentinel node dissection wasn’t performed because it was arguable the benefit for the patients taking into account the location and number of hot spots.

All this 4 patients are free of disease and any of them have developed recurrences. In 14 (56%) patients all the sentinel nodes were identified and removed. Three (3/14 = 21%) patients had positive sentinel nodes (1 in the ipsilateral axilla, 1 internal mammary and 1 in the contralateral axillary). One patient had 2 positive contralateral axillary sentinel nodes so an ALND was performed with no additional positive nodes. No surgical complications were observed.

Conclusions: In patients who had a previous ALND who develop a breast cancer recurrence, the SPTC-CT might show the exact location of aberrant hot spots but, some of the identified as hot spots may not
be lymph nodes. We must consider the exact location of the hot spot and the likelihood of false positives to avoid additional morbidity on the procedure.

**P3-07-46**

Accuracy of SPIO-Enhanced MR Imaging Alone for the Diagnosis of Sentinel Node Metastases in Patients with Breast Cancer.

Motomura K, Nakahara S, Ishitobi M, Komoike Y, Koyama H, Inaji H, Horinouchi T, Nakanishi K. Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka, Japan

**Background:** Superparamagnetic iron oxide (SPIO)-enhanced MR imaging has been reported to be promising for the detection of metastases in sentinel nodes localized by CT lymphography in patients with breast cancer (Motomura, Ann Surg Oncol 2011). The current SPIO technique involves imaging before and after contrast administration. This study evaluated the accuracy of SPIO-enhanced MR imaging alone without unenhanced imaging.

**Methods:** This study included 120 patients with breast cancer demonstrating clinically negative nodes. Sentinel nodes were identified by CT lymphography, and MR imaging of the axilla before and 18-24 hr after interstitial administration of SPIO was performed. A node was considered non-metastatic if it showed a homogenous low signal intensity and metastatic if there was an absence of low signal intensity either in the entire node or in a focal area on SPIO-enhanced MR imaging. The diagnostic accuracy of the SPIO-enhanced imaging alone was compared with that of combined unenhanced and SPIO-enhanced imaging.

**Results:** The mean number of sentinel nodes identified by CT lymphography was 1.2 (range 1-3). Pathologic evaluation demonstrated that 28 (23.3%) of 120 patients showed metastasis to at least one node. One false negative result was added when the evaluation was based solely on SPIO-enhanced MR imaging. Consequently, the sensitivity decreased from 84.0% to 80.0% and the accuracy decreased from 92.2% to 88.3%, respectively, in the SPIO-enhanced MR imaging alone. However, the differences in sensitivity and accuracy between SPIO-enhanced MR imaging alone and the combined unenhanced and SPIO-enhanced imaging were not significant (McNemar's test: p=1.0). The specificity of enhanced imaging alone and that of combined unenhanced and SPIO-enhanced imaging were both 90.5%.

**Conclusions:** A single MR imaging examination performed after SPIO administration can be used for accurate diagnosis of sentinel node metastases, and thus reduce the time and cost of imaging.

**P3-07-47**

Validated Nomogram To Predict Sentinel Lymph Node Positivity in Breast Cancer Patients.

Kaplan R, Qiu Q, Monni S, D’Alfonso T, Shin SJ. The New York-Presbyterian Hospital - Weill Cornell Medical College, New York, NY

Background: Sentinel lymph node (SLN) biopsy is commonly used to assess axillary lymph node status in breast cancer patients. Intraoperative (frozen section) evaluation of SLN may lead to immediate axillary dissection if the lymph node is found to be positive. In light of the recent outcomes of the Z00011 trial, the use of intraoperative evaluation for SLN should diminish since only select patients appear to benefit from axillary dissection. We have previously reported that tumor size and lymphovascular invasion (LVI) strongly predict SLN positivity in a cohort of 350 breast cancer patients [Modern Pathology (2011) 24, 30A]. From this test cohort, we developed a nomogram which could help identify patients more likely to have sentinel lymph node positivity. The aim of this study was to validate the efficacy of this nomogram by applying a new, non-overlapping cohort of breast cancer patients who had undergone sentinel lymph node sampling. Materials and Methods: 93 breast cancer patients aged 29-82 (mean:59) who underwent excision (54, 58%) or mastectomy (39, 42%) with a SLN biopsy were studied. Patients who had undergone neoadjuvant chemotherapy were excluded. Clinicopathologic parameters such as number of sentinel lymph nodes removed and/or positive, number of non-sentinel lymph nodes removed and/or positive, tumor size, histologic type/grade, presence of lymphovascular invasion, biomarker expression (ER,PR, HER-2/neu) and multifocality were recorded. Statistical analysis was performed to identify which variables correlated with SLN positivity in this validation cohort. In addition, the probability of SLN positivity for each case was determined by using the nomogram and this value was then compared to the patient’s true SLN status. Results: When analyzed separately, tumor size (p<0.03) and LVI (p<0.01) were variables that were significantly associated with SLN positivity in both the test and validation cohorts. Employment of the nomogram demonstrated that 60% of patients who were predicted to have a positive SLN with at least 0.5 probability were truly positive by histologic and immunohistochemical examination. Moreover, 89% of patients who were predicted to have a negative SLN with at least 0.8 probability were truly negative by histologic and immunohistochemical examination. Conclusions: We conclude that the utility of this nomogram prior to surgery can help predict SLN positivity. This can serve as a complimentary adjunctive tool to better select patients who will likely need intraoperative evaluation of their sentinel lymph node due to their higher risk of sentinel node positivity and thus, benefit from the option of immediate axillary dissection at that time.

**P3-07-48**

Axillary Imaging with Dynamic MRI Following Subcutaneous Injection of Superparamagnetic Iron Oxide Nanoparticles.

Douek M, Johnson L, Parikh J, Charles-Eduards G, Hall-Craggs M. King’s College London, London, United Kingdom; Guy and St Thomas’ Hospitals, London, United Kingdom; University College Hospital, London, United Kingdom

Background: Surgical axillary staging with sentinel node biopsy in clinically node negative patients is standard of care in the management of breast cancer. However, sentinel node biopsy is associated with morbidity including a 5% risk of lymphoedema. Superparamagnetic iron oxide (SPIO) enhanced axillary MRI is a promising novel imaging modality that could be used to characterize sentinel nodes non-invasively. We evaluated subcutaneous SPIO enhanced axillary MRI for pre-operative axillary imaging.

Material and methods: Patients scheduled for sentinel node biopsy as part of surgical management of early breast cancer were invited to undergo pre-operative axillary MRI. All images were acquired on a 1.5T scanner using a surface coil. The initial 7 scans were acquired on a Siemens Avanto and the later scans on an Achieva MRI scanner (Philips, Best, Netherlands). Following a T2-weighted morphological scan, patients were injected with 2ml of SPIO (4ml in the final 3 patients) subcutaneously into the circumareolar margin in the upper outer quadrant of the affected breast. Post injection, a slightly T2*-weighted dynamic scan was performed (gradient echo, TE = 1.53ms, TR=2.9ms, flip angle 7 degrees, 3mm slice thickness). In addition to the dynamic scan, in 16 patients, a T2 mapping sequence was performed at 10minutes and 120 minutes post injection (turbo spin echo, 8 equi-spaced TE's from 10 to 80ms, TR=2136ms, 3mm slices with an in plane resolution of 1.4x1.4mm). Image analysis was...
undertaken using Osirix (v3.8, 64-bit). Two consultant radiologists experienced at reading breast and axillary MRI reported all scans. Results: A total of 23 patients underwent axillary MRI with subcutaneous SPIO. Of these 18 patients (78%), uptake of SPIO was seen in sentinel nodes and lymphatic tracts. At least 1 sentinel node was identified in 17 patients (74%). A total of 106 nodes were identified (4.6 ± 1.7 nodes per patient) and of these 40 demonstrated a significant drop in signal intensity following SPIO injection (1.7±1.3 nodes per patient). All 3 involved nodes were seen to contain a metastatic deposit on MRI.

Discussion: Axillary MRI with subcutaneous SPIO injection is a robust method for imaging sentinel nodes. The normal node count of the axillary basin is lower than expected on histology, suggesting that only the larger nodes and those that take up SPIO are visualized on MRI.

P3-08-01
Effects of an Integrated Yoga Program on Mood States, Distress, Quality of Life, Diurnal Cortisol Rhythms and Natural Killer Cell Counts in Metastatic Breast Cancer Survivors.
Gopinath SK, Rao RM, Sanjeevarao VH, Diwakar RB, Basavalingiaah AS, Patil S, Raghuram N, Rama Rao N, Usharani RM. HCG - BIO Super Speciality Centre, Bangalore, Karnataka, India; Swami Vivekananda Yoga Anusandhana Samsthana, Bangalore, Karnataka, India

Background and objectives: Metastatic breast cancer patients experience tremendous psychological distress due to treatment, disease and uncertainty of their survival. In this study we evaluated the effects of an integrated yoga program versus supportive counseling in advanced breast cancer survivors.

Methods: Ninety one Metastatic breast cancer survivors (group mean age 50.54 yrs ± 8.53 yrs) registered in hospital based cancer registry were recruited if they satisfied selection criteria and gave written consent for participation in the study. The study was approved by the institutional review board. Subjects were randomized to either receive yoga intervention (n=45) or supportive therapy (n=46) counseling as a standard of care for 3 months of intervention period. Subjects were assessed at baseline and after intervention for mood states using hospital anxiety and depression scale, sleep quality using Pittsburg Insomnia rating scale, quality of life using EORTC QoL C30 for breast and perceived stress using perceived stress scale. Saliva samples were collected between 0800hrs to 01000hrs for NK cell enumeration using flow cytometry. 35 subjects in yoga and 31 group compared to controls. There was significant decrease in 0600 hrs cortisol within the yoga group (p<0.001), role function (p=0.03) alone. There was a significant drop in signal intensity following SPIO injection (1.7±1.3 nodes per patient). All 3 involved nodes were seen to contain a metastatic deposit on MRI.

Results: There was a significant decrease in anxiety (p < 0.001), depression (p < 0.001), perceived stress (p = 0.01), fatigue severity (p < 0.001) and interference (p < 0.001) in yoga group compared to controls post intervention. There was a significant improvement in emotional function (p < 0.001), role function (p = 0.03) and cognitive function (p < 0.001) and global quality of life (p < 0.001) in yoga group compared to controls. There was significant decrease in 0600 hrs cortisol within the yoga group (p =0.03) alone. There was a significant increase in Natural killer cell percent in yoga group (p =0.03) compared to controls after intervention. Conclusion: The results suggest biobehavioral effects of yoga intervention could possibly improve quality of life, reduce psychological distress and modulate abnormal cortisol profiles and immune responses in metastatic breast cancer patients.

P3-08-02
Evaluating the Impact of Educational Material on Anastrozole Treatment Adherence – The Final Results of the ARTEMIS Study.
Nogaret J-M, Coibion M, Neven P, Soepenberg O, Graas M-P, Deschamp V, Vanlerberghe T, Jules Bordet Institute, Brussels, Belgium; CHC St-Vincent, Rocourt, Belgium; University Hospital Leuven, Leuven, Belgium; MariaZiekenhuis, Noord-Limburg, Belgium; CHC Saint-Joseph, Liège, Belgium; AstraZeneca Benelux, Brussels, Belgium

Rationale: Breast cancer (BC) is the most common cancer in European women. Compliance with the prescribed dosing regimen is essential for optimal effectiveness of cancer treatment. While adjuvant endocrine therapy reduces the risk of BC recurrence, only limited information is available regarding patient adherence to this type of treatment. Electronic monitoring of patients’ dosing histories has demonstrated the frequently irregular drug intake of patients. The ARTEMIS study (Arimidex Therapy compliance Electronic MonitorIng System, NCT00936442) evaluated the impact of educational material on the adherence to adjuvant treatment with anastrozole (Ax, Arimidex®).

Methods: This randomized (1:1), open-label, parallel-group study in 5 centers in Belgium had two treatment groups: GpA-Standard Treatment, GpB-Standard Treatment+Education. All patients received a 12 month follow-up of Ax treatment according to current clinical practice. Patients in GpB also received additional educational material on a regular basis (9 mailings). Eligible patients were postmenopausal women with hormone sensitive early BC prescribed Ax (<13 weeks duration). All patients received Ax (1mg orally/day) in a Medication Event Monitoring System (MEMS® AARDEX Ltd) that electronically records bottle openings. The 500 ml capacity contained medication for 6 months treatment to avoid additional clinical visits to resupply medication. The sample size was based on the primary objective: adherence to Ax (the daily proportion of patients taking Ax). Secondary variables included persistence with Ax (the estimated length of time during which the medication is taken), execution of the Ax regimen and reasons for treatment discontinuation.

Results: 107 patients (mean age 61.4±9.9 years) were enrolled: 54 in GpA, 53 in GpB. 16 patients (9 GpA, 7 GpB) discontinued prematurely based on the case report form with the MEMS system indicating that a further 4 patients (3 GpA, 1 GpB) discontinued. Overall, the adherence rate was high and decreased significantly over time (p=0.0061). There was no significant difference in adherence rate between the two groups (p=0.2358) with the daily average probability of correct intake 80.2±4.16% (GpA) and 86.7±4.94% (GpB). There was a trend for the persistence rate after 12 months to be better in GpB (84.7±5.0%) compared to GpA (77.8±5.7%; p=0.0782). An exploratory analysis showed a significant association between overall persistence and increasing postmenopausal age (Exp(coef)=0.942; p=0.038). The execution of the dosing regimen improved over time (p=0.0126) and was similar throughout the study for both groups (GpA: 96.5±3.1%, GpB: 94.0±3.1%; p=0.1674). Side effects (5 patients, 4.7%) were the most common reason for early discontinuation (GpA: 1 patient, GpB: 4 patients) but overall side effects were low.
Conclusion: Educational material by mail did not significantly affect patient adherence to anastrozole. Persistence with treatment was associated with the post-menopausal age of the women and showed a trend for improvement in those receiving educational material. Since anastrozole treatment normally lasts 5 years, a longer-term follow-up study may provide better insight into the usefulness of MEMS on treatment compliance.

P3-08-03
Exercise Increases Soluble Vascular Endothelial Growth Factor Receptor-1 (sFlt-1) in the Circulation of Adult Women.
Makey K, Patterson SG, Robinson J, Loftin M, Waddell DE, Miele L, Chinchar E, Huang M, Smith AD, Weber M, Gu J-W. University of Mississippi Medical Center, Jackson, MS; University of Mississippi, Oxford, MS

Background: Physical inactivity increases the risk of several different cancers, including breast cancer. Soluble fms-like tyrosine kinase-1 (sFlt-1) is an extra-cellular Ig-like domain of the VEGF receptor-1 that is released into the extracellular space and circulation where it inhibits the activities of VEGF. Over-expression of sFlt-1 has been shown to inhibit ovarian tumor growth in gene therapy experiments. The present study tests the hypothesis that exercise can increase sFlt-1 in the circulation of adult women.

Material and Methods: 63 African American and Caucasian adult woman volunteers aged 18-44 were enrolled into a prospective exercise study. All the participants walked on a treadmill for 30 minutes at a moderate intensity (40-60% heart rate reserve), and oxygen consumption (VO2) was quantified by utilizing a metabolic cart. Blood samples were collected before and immediately after exercise. The exercise test was conducted between the first and seventh day of the participant’s menstrual cycle. The plasma concentrations of sFlt-1, unbound VEGF, and endostatin were measured using ELISA kits from R and D Systems.

Results: Plasma levels of sFlt-1 were 67.8±3.7 pg/ml immediately after exercise (30 minutes), significantly higher than basal levels of 54.5±3.3 pg/ml before exercise (P=0.01; n=63). The % increase in sFlt-1 levels before and after exercise in adult women was 54%. There was no significant difference in the % increase of sFlt-1 levels between African American and Caucasian groups (P=0.5334). There was no significant difference in plasma levels of endostatin before (92.4±4.4 ng/ml) and immediately after (93.8±4.4 ng/ml; P=0.8216) exercise. The basal plasma levels of unbound VEGF (21.5±4.3 pg/ml) were similar to the plasma levels of VEGF (22.5±4.6 pg/ml; P=0.8652) immediately after exercise.

Discussion: We previously reported that plasma levels of sFlt-1 significantly increased 30 minutes after exercise in adult men, in which plasma levels of unbound-VEGF significantly decreased and plasma levels of endostatin significantly increased 2 hours after exercise. Until now, there has been no data on whether exercise increases plasma sFlt-1 levels in women. Our study is the first to show that exercise in adult women significantly increases plasma levels of sFlt-1. VEGF pathways have both autocrine and paracrine effects for promoting breast cancer progression. Previous studies have demonstrated that sFlt-1 inhibits the activities of VEGF and suppresses ovarian tumor growth in mice. Exercise-induced plasma levels of sFlt-1 could be an important clinical biomarker to explore the mechanisms of exercise training in reducing breast cancer progression. The sFlt-1 is produced in the microvascular and macrovascular endothelial cells that exist throughout the skeletal muscle tissues. It is therefore plausible that release of sFlt-1 from the skeletal muscles into the circulation might be due to exercise-dependent reductions in oxygen tension in the skeletal muscle. In future studies, we will determine whether sFlt-1 can be released directly from the exercised muscle.

P3-08-04
“How Important Is This for Me?” – The Role of Necessity Beliefs as Determinants of Breast Cancer Prevention Intentions among High-Risk Women.
Verma S, Paquet L, Stacey D, Davis I, Bedard M, Lowry S, Ianni L. The Ottawa Hospital Cancer Centre, Ottawa, ON, Canada; Carleton University, Ottawa, ON, Canada; University of Ottawa, Ottawa, ON, Canada

Background: Women at elevated risk for breast cancer (BC) face complex risk management decisions. Understanding the determinants of pre-counseling risk management intentions would be useful to clinicians in helping high-risk women make their decisions. Across several medical conditions, the Necessity-Concerns Framework (NCF) has emphasized the role of patient’ beliefs about interventions offered to them as central to decision-making. In the NCF, beliefs are conceptualized as perceived personal need for an intervention (necessity) and as concerns about its perceived negative effects. To date, the NCF has not been applied to investigate decision-making in the high-risk setting. Our objective was to use the NCF to describe the risk management beliefs of high-risk women prior to initial consultation.

The relative importance of BC fear, perceived susceptibility to BC and necessity-concerns beliefs in predicting prevention intentions was also examined. Methods: A survey was sent to patients prior to their first risk consultation. BC fear was assessed with Champion BC Fear Scale and perceived susceptibility was measured by asking women to rate their likelihood of developing BC (0 = definitely will not get it to 100 = definitely will get it). For each of screening, lifestyle modifications, pharmacoprevention (PP) and prophylactic surgery (PS), women rated (1 = strongly disagree to 5 = strongly agree) their need for the option (e.g., my health in the future will depend on this option, this option will protect me from becoming worse), and its negative consequences (e.g. I worry about the long-term effects of this option, this option will disrupt my life). Intentions were assessed by asking how strongly (1 = definitely not to 5 = Definitely Yes) they intended to adopt each option. The planned sample size is 100 women and recruitment is on-going. We report data from 44 women who have completed the questionnaire. Results: The majority intended to adopt screening (92%) and lifestyle (91%) compared to PP (23%) or PS (18%). Screening and lifestyle were associated with stronger intentions, higher perceived need, and lower concerns than PP and PS (all t-tests, p < 0.0001). Hierarchical regression analyses revealed that perceived need predicted intentions for each option (all ps < 0.005, except for screening, p < .1) whereas concern beliefs were only correlated with PP intentions (β = -0.398, p < .005). Fear of BC was related to PP intentions (β= -0.401, p < .005) and to intentions to remain smoke-free (β = -0.341, p < .05). Perceived susceptibility did not contribute to intentions. Conclusions: Our preliminary findings are promising and suggest that the NCF is useful for understanding decision-making in the high-risk setting. The findings emphasize the importance of necessity beliefs in BC risk management decisions. For the group as whole, across each risk management option, the strongest predictor of intentions was a women’s perception of her personal need for the option. Concerns about negative effects and BC fear played only a minor role and perceived susceptibility did not predict intentions. These observations stress the importance of making screening or prevention recommendations hand in hand with the individual beliefs and concerns.
P3-09-01
Change in Carbohydrate Intake and Breast Cancer Prognosis. Emond JA, Patterson RE, Pierce JP. UCSD Moores Cancer Center; La Jolla, CA; San Diego State University, San Diego, CA
Breast tumors over express insulin-like-growth-factor receptors (IGF-1R), and levels of expression may be inversely related to tumor grade. Dietary factors, particularly carbohydrate intake, may stimulate activation of IGF-1R and affect prognosis.
METHODS: Data are from N=2,651 women in the Women’s Healthy Eating and Living (WHEL) Dietary Intervention trial, a plant based intervention trial that did not have a carbohydrate goal. All women were diagnosed with breast cancer within the previous 4 years. Carbohydrate dietary intake data were extracted from multiple 24-hour dietary recalls at study entry and one year, and were compared by recurrence status. Time to recurrence was modeled on year-one change in carbohydrate intake adjusted for baseline intake, menopausal status, and disease, treatment, and study characteristics.
RESULTS: Baseline carbohydrate intake was 233 g/day. Women who recurred had a mean increase in carbohydrate intake over the first year, compared to those who did not recur (2.3 ±2.7 g/day; p=0.188). Change in starch intakes accounted for 48% of the change in carbohydrate intake (R-squared: 48%; p<0.001). Baseline starch intake did not differ by recurrence status (95.8 g/day; p=0.219). Mean year-one change in starch intake was -4.1 g/day among women who recurred vs -8.7 g/day among women who did not recur (p=0.015). Year-one change in starch intake was independent of the study intervention (p=0.326). In the adjusted model, a 5-g/day increase in starch related to a 3% increased risk of recurrence (HR=1.03; 95% CI 1.01 – 1.06; p=0.017). The increased risk was limited to women diagnosed with low grade tumors (Table 1).

<table>
<thead>
<tr>
<th>Year-one change, 5g/day</th>
<th>Overall</th>
<th>Stratified By Primary Tumor Grade</th>
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<td>HR (95% CI); p-value</td>
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<td>-1.05 (1.01-1.06)</td>
<td>0.017</td>
<td>1.05 (1.01-1.09); p=0.007</td>
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<td>1.00 (0.96-1.04); p=0.981</td>
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DISCUSSION: Dietary modifications targeting starch intake warrant further investigation as a preventive measure against breast cancer recurrence.

P3-09-02
Intermittent Dietary Carbohydrate Restriction Enables Weight Loss and Reduces Breast Cancer Risk Biomarkers.
Harvie M, Wright C, Pegington M, Mitchell E, Evans DG, Jebb S, Clarke R, Goodacre R, Dunn W, Mattson M, Howell A. University Hospital of South Manchester, Manchester, United Kingdom; MRC Human Nutrition Research Group, Cambridge, United Kingdom; University of Manchester, Manchester, United Kingdom; National Institute of Aging, Baltimore
Background: Energy restriction is a potential strategy for breast cancer prevention but is difficult to achieve and maintain. We found that intermittent energy restriction (2 days strict dieting week) is comparable to the standard approach of moderate daily restriction for weight loss and marginally better for improving insulin sensitivity, but no easier to follow1. In this follow on study we wished to test whether 2 novel intermittent low carbohydrate/low energy diets were feasible and easier to follow than a standard daily energy restriction. Design: Randomised comparison of 3 dietary types over 4 months in 115 overweight or obese (mean body mass index 31.0 ±5.3 SD) kg/m² women at increased risk of breast cancer (lifetime risk > 1 in 6).
Diets:
1. A restricted low carbohydrate diet (RLCD): 650 kcal and <50g carbohydrate / day for 2 days per week
2. Ad lib low carbohydrate diet (ALCD): <50g / day for 2 days per week with other food types (e.g. protein) ad lib
3. A standard daily restricted Mediterranean diet (DRMD): ~1500kcal / day for 7 days per week

Methods: Weight, anthropometrics, blood markers for breast cancer; insulin resistance, oxidative stress markers, leptin, adiponectin, lipids, inflammatory markers IGF-1 were assessed at baseline, 1, 3 and 4 months.
Results: 88/114 completed the study (77%, drop outs 6 RLCD, 8 ALCD 12 DRMD). Last observation carried forward analyses show both intermittent low carbohydrate diets were superior to standard daily restriction for reducing weight and body fat: mean (95% confidence interval [CI]) change in body fat for RLCD was -4.3 (-5.6 to -3.0) kg, for ALCD -4.1 (-5.2 to -3.1) kg vs. -2.4 (-3.4 to -1.2) kg for DRMD (P value for difference between groups = 0.02). The intermittent groups had greater improvement in insulin resistance: mean (95% CI) change for RLCD was -22 (-35 to -11) %, ALCD -14 (-27 to -5%) % vs. -4 (-16 to 9) % for DRMD (P = 0.02). Other biomarkers are being assayed currently.
Conclusion: Greater weight loss, fewer drop outs and greater reductions in insulin resistance with the novel intermittent low carbohydrate diets indicate that these are alternative approaches for energy restriction for potentially reducing risk of breast cancer and other diseases.
Reference:
1 Harvie MN, Pegington M, Mattson MP, Frystyk J, Dillon B, Evans G et al. The effects of intermittent or continuous energy restriction on weight loss and metabolic disease risk markers: a randomized trial in young overweight women. Int J Obes (Lond) 35; 714-27, 2011. This study is funded by the Genesis Breast Cancer Prevention Appeal: www.genesisuk.org

P3-09-03
Long-Chain Polynsaturated Fatty Acid Intake and Its Relationship to Long-Chain Polynsaturated Fatty Acids in Serum, Red Blood Cells and Breast Tissue.
Harvey KE, Li S, Carlson SE, Sullivan DK, Klemp JR, Kimler BF, Fabian CJ. University of Kansas Medical Center; Kansas City, KS
Background: Long chain omega-3 (n-3) polyunsaturated fatty acids (LCPUFA) have anti-inflammatory effects and are able to counteract the effects of the pro-inflammatory omega-6 (n-6) fatty acids such as arachidonic acid (AA) by substituting for the n-6 fatty acids in triglycerides (TG) and phospholipids (PL). Several pre-clinical, observational, and case control studies suggest that intake or tissue content of n-3 LCPUFA such as eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), relative to intake or tissue content of long chain n-6 fatty acids such as AA may be associated with reduced risk of breast cancer. The goal of this study was to determine the relationships between dietary intake of fatty acids, tissue levels of fatty acids, and breast tissue biomarkers for risk of breast cancer.
Methods: Women (n=74) were recruited from a clinic in which women at increased risk for breast cancer had breast tissue acquired by random periareolar fine needle aspiration (RPFNA). Breast epithelial cells were assessed for cytomorphology and proliferation (Ki-67 immunochemistry). Fatty acid dietary intake was assessed with the National Cancer Institute Diet History Questionnaire. Plasma, erythrocyte, and breast specimens were processed for membrane PL and TG and analyzed for individual fatty acids by gas liquid chromatography.
Results: Total intake of n-3 PUFA was 1.1 ± 0.5 g/d, and the ratio of EPA+DHA:AA was 0.1:1.0 (n=66). Dietary n-3 LCPUFA correlated...
with n-3 LCPUFA in both plasma and erythrocyte PL (n=62). Breast epithelial cell number, Masood cytomorphology score, and percent Ki-67 positive cells were higher in RPFNFA specimens which exhibited cytologic atypia compared to those which did not (n=74; p=0.001, Mann-Whitney Test). Subjects with atypia consumed less dietary n-3 PUFAs (n=66, p=0.020), had lower plasma and erythrocyte PL and plasma TG EPA, DHA, total n-3, and EPA+DHA:AA (n=70; p=0.05). In breast tissue TG, the ratio of n-3:n-6 was also lower in subjects with atypia (n=40; p=0.025).

Conclusions: Overall, women in this high risk cohort consumed very low amounts of n-3 LCPUFAs. Dietary intake of n-3 LCPUFA was related to levels of n-3 LCPUFA in erythrocyte and plasma PL. Given the association of low levels of n-3 fatty acids with cytologic atypia (a known risk factor for breast cancer development), an intervention to increase n-3 fatty acids and n-3:n-6 ratios has merit and clinical trials in high risk women have been initiated.

P3-09-04
Comparative Preventive Efficacy of Aqueous Extracts from Lycium Barbarum Bark and Fruit on Estrogen Receptor Positive Human Mammary Carcinoma MCF-7 Cells.
Telang NT, Li G, Sepkovic DW, Bradlow LH, Wong GGY, Telang NT. Palindrome Liaisons, Montvale, NJ; American Institute for Chinese Medicine, New York, NY; Hackensack University Medical Center, Hackensack, NJ

Background: Chemotherapy, selective estrogen receptor modulators and aromatase inhibitors represent major treatment options for estrogen receptor positive (ER+) clinical breast cancer. These modalities frequently exhibit acquired resistance and adverse systemic toxicity. Natural herbs are increasingly being used in the integrative support of breast cancer patients undergoing conventional treatment modalities and for prevention purposes. We previously reported favorable findings for the fruit of Lycium barbarum (LB), known commonly as goji berry, in the prevention for ER+ breast cancer (Nutrition & Cancer 61:408-414, 2009). The present study compares the preventive efficacy of non-fractionated aqueous extracts from the bark of LB (LBB) and the fruit of LB (LBF) on a cell culture model for ER+ breast cancer.

Material and Methods: The ER+ human mammary carcinoma derived MCF-7 cells represented the model. Anchorage dependent growth, anchorage independent colony formation and cellular metabolism of 17β-estradiol (E2) represented the mechanistic bases for efficacy. Results: MCF-7 cells grown in serum depleted medium retained their responsiveness to E2, exhibiting E2 stimulated promotion of anchorage independent colony formation and cellular metabolism. LBB produced a 6.8 fold increase in 2-hydroxyestrone (2-OHE1), a 40% decrease in 16α-hydroxyestrone (16α-OHE1) and a 3.7 fold increase in estriol (E3) formation. The corresponding values for LBF were 3.9, 33 and 10.5. LBB treatment resulted in a 16.3 fold increase in estriol (E3) formation. The corresponding values for LBF were 3.9, 33 and 10.5. LBF treatment produced a 6.0 fold increase and a 2.9 fold increase, respectively, in these endocrine biomarkers. These data suggest that the preventive efficacy of LBB may predominantly be due to up-regulation of the anti-proliferative 2-OHE1 formation, while accelerated conversion of the pro-mitogenic 16α-OHE1 to the mitogenically inert E3 is the case with LBF.

Discussion: Growth inhibitory profiles of LBB and LBF may in part be due to their distinct chemical composition and their complementary actions on E2 metabolism. The superior sensitivity of LBB relative to LBF, together with its non-toxic nature, suggests the feasibility of its use in the prevention of human breast cancer. A prevention trial to assess its efficacy and to establish relevant dose schedule is warranted. This study validates a mechanism based approach to identify and prioritize efficacious herbal extracts for the prevention of ER+ breast cancer.

P3-09-05
Understanding the Role of Estrogen in Sex Differences in Adipocyte Biology for Cancer Prevention.
Stubbins RE, Holcomb VB, Hong J, Nunez NP. The University of Texas at Austin, Austin, TX

Background: Evidence shows that obesity increases the risk and mortality of many cancers, specifically breast cancer in post-menopausal women. Epidemiological studies demonstrate that males are at an increased risk for developing obesity, diabetes, and cancer compared to females, and after menopause, females mimic the males in their susceptibility to the above diseases. Furthermore, it has been established that obesity increases systemic and adipose tissue inflammation and oxidative stress, which is known to augment cancer progression. However, the role of estrogen in regulating these metabolic processes in adipose tissue is unclear; therefore, our objective is to determine the role of estrogen in adipose tissue morphology, inflammation and oxidative stress.

Methods: To determine the role of estrogen in gender differences in the susceptibility to obesity we used C57BL/6J mice (15/group): 1) males 2) nonovariectomized females 3) ovariecetomized females and 4) ovariecetomized females supplemented with estrogen, which were randomized to the following diets: 30% calorie-restricted, low-fat or high-fat diet. We measured weight gain, percent body fat, abdominal adipose tissue, and adipocyte size. Additionally, we assessed markers of adipose tissue inflammation, DNA damage, and oxidative stress.

Results: Male mice were more susceptible to obesity than female mice. Removal of the ovaries eliminated the protection to obesity and estrogen supplementation restored this protection in females. In the low-fat and high-fat diet groups, male and ovariecetomized female mice gained more abdominal adipose tissue due to increased adipocyte size compared to nonovariectomized female mice and ovariecetomized female mice supplemented with estrogen. In the mice consuming the high fat diet, the enlarged adipocytes observed in the male and ovariecetomized female mice were accompanied with crown-like structures surrounding necrotic adipocytes and F480 positive macrophages, suggesting macrophage infiltration. To determine if there were sex differences in oxidative stress, we stained adipose tissue with γH2AX. Results suggest that nonovariectomized female mice and ovariecetomized female mice supplemented with estrogen have less oxidative stress compared to males and ovariecetomized females. Additionally, our results show that nonovariectomized female mice and ovariecetomized female mice supplemented with estrogen have less oxidative stress compared to males and ovariecetomized females. In ovariecetomized females, estrogen supplementation has a protective effect against obesity, adipose tissue inflammation and oxidative stress.

Conclusion: Male mice are more susceptible to the obesogenic effects of high fat diets compared to nonovariectomized female mice. In ovariecetomized females, estrogen supplementation has a protective effect against obesity, adipose tissue inflammation and oxidative stress.
stress. Our future studies will determine the mechanisms by which estrogen protects female mice from adipose tissue inflammation and oxidative stress, whether it is a direct or indirect effect. Determining the role of estrogen on the above key metabolic processes related to obesity is necessary to develop effective strategies for cancer prevention specifically in post-menopausal females.

**P3-09-06**

Changes of Serum Vitamin D According to the Breast Cancer Treatment.

**Kim HJ, Yi OY, Koh BS, Yu JH, Lee JW, Son BH, Ahn SH. Asan Medical Center, Seoul, Korea**

**Background**

Vitamin D deficiency is associated with increased breast cancer risk and decreased breast cancer survival. The purpose of this study was to determine the effect of breast cancer adjuvant treatment to the vitamin D status, as measured by the serum hydroxyvitamin D (25OHD) in breast cancer patients.

**Patients and Methods**

For 589 patients who was diagnosed as a non metastatic breast cancer in 2009 at the asan medical center, blood was prospectively analyzed in batches for serum 25 OHD level at basal and at 6 and 12month. We excluded the patients who took a vitamin D supplementation and got a neoadjuvant chemotherapy. Vitamin D sufficiency was defined as serum as 30ng/ml or greater, insufficiency as 20 to 29 ng/ml and insufficiency as less than 20ng/ml.

**Results**

At baseline, mean serum 25OHD was greater in summer (April to Oct) than Winter(Nov to May ) (28.2ng/ml vs 32.9ng/ml respectively, p=0.000). The patients who did not get a chemotherapy and anti-hormonal therapy as baseline, the patient with chemotherapy showed decreased serum 25OHD level than who without chemotherapy in 6 month but not in 12 month (p=0.003, vs p=0.156 respectively). The patients who had taken anti-hormonal therapy showed significant increasing serum 25OHD in 6 month and 12 months (p=0.000 both).

For the patients who got both chemotherapy and anti-hormonal therapy, the changes of serum 25OHD level is smaller than the patients who got a chemotherapy only.

For the patients who got a chemotherapy, 57% of patients were vitamin D sufficient at baseline, but 27% of patients in 6 month and 49% in 12 month (p=0.001).

**Conclusion**

Vitamin D status was worsen during chemotherapy but recovered after chemotherapy. Anti hormonal therapy make the serum vitamin D level increased. The translational research about the effect of chemotherapy and anti-hormonal therapy to the vitamin D status should be warranted.

**P3-10-01**

Alternative Dosing Regimens with the EGFR Inhibitors (Gefitinib and Lapatinib) in Mammary Cancer Models: Prevention and Therapeutic Efficacy.

**Lubet RA, Bode AM, Szabo E, Grubbs CJ. National Cancer Institute, Bethesda, MD; Hormel Institute, Austin, MN; University of Alabama at Birmingham, Birmingham, AL; Milwaukkee, WI**

The EGFR inhibitors are effective in treatment of lung and pancreatic cancers (erlotinib, gefitinib) and Neu overexpressing breast cancer (lapatinib) in humans; as well as preventing multiple cancers in animal models. However, the development of toxicities (Iressa, skin rashes; Lapatinib, diarrhea) limit their potential use in prevention; and perhaps even in an adjuvant setting. We examined whether alternative dosing regimens which might reduce toxicity would still achieve preventive and therapeutic efficacy. Female Sprague-Dawley rats were administered a single IV dose of methylnitrosourea (MNU) at 50 days of age. In a prevention study, MNU treated rats administered Gefitinib daily (10 mg/kg BW/day, 7x/week) or Gefitinib (70 mg/kg BW, 1x/week) beginning 5 days after MNU resulted in 94 and 75% reductions, respectively, in cancer multiplicity. Simultaneous measurements of tumor load (number of tumors x tumor weight) showed that both regimens resulted in greater than a 90% decrease. In the therapeutic study (initiating treatment when animals developed a small palpable cancer), both regimens were again highly effective. A prevention study was also performed with Lapatinib (75 mg/kg BW/day, 7x/week or 525 mg/kg BW, 1x/week). While the daily dose reduced cancer multiplicity 90%, the weekly dose caused a 70% reduction. Finally, we examined the effects of daily or weekly dosing with Iressa (100 mg/kg BW/day, 5x/week or 500 or 250 mg/kg BW, 1x/week) in an ER mouse model (using MMTV-Neu p53−/− mice). This study showed that while daily dosing with Iressa decreased tumor multiplicity roughly 80%, weekly dosing at either dose caused roughly a 50% decrease. These data show that even a significant alteration in the dosing of Gefitinib (EGFR 1) or Lapatinib (EGFR 2/1) still resulted in a large reduction in mammary cancers. The important clinical question will be whether this altered dosing will diminish the toxicities associated with these agents. We cannot determine this in animal models since, at the effective doses employed, the typical toxicities observed in humans are not observed. Supported by NC1 contract number HHSN261200433001C.

**P3-10-02**

Gene Expression Changes in Methylnitrosourea (MNU)-Induced ER+ Mammary Cancers Following Short-Term Treatment of Rats with the Aromatase Inhibitor Vorozole.

**Lubet RA, Grubbs CJ, Bode A, You M, Lu Y. National Cancer Institute, Bethesda, MD; University of Alabama at Birmingham, Birmingham, AL; Hormel Institute, Austin, MN; Medical College of Wisconsin, Milwaukee, WI**

Aromatase inhibitors have proven to be highly effective in both therapy and prevention of ER+ breast cancer. Vorozole (R83842), a high affinity competitive inhibitor of aromatase (similar to letrozole and anastrozole) showed strong activity in early clinical trials against ER+ breast cancer. Furthermore, vorozole was highly effective in the prevention and therapy of ER+ cancers in the MNU-mammary cancer model (Lubet, et al., Carcinogenesis 19, 1345, 1998). In the present study, rats bearing mammary cancers induced by MNU were exposed to vorozole (1.25 mg/kg BW/day) for 5 days. Global gene expression analysis showed that 162 genes were down-regulated and 180 genes up-regulated in cancers treated with vorozole (p < 0.05 and fold change > 1.5). The genes modulated by vorozole were compared with two additional sets of data. First, thirty-two genes and a number of pathways exhibited significantly concordant changes with aromatase inhibitors both in the animal model and in at least three of four published human data sets. In particular, differentially expressed genes enriched in the cell cycle pathway that were related to chromosome condensation in prometaphase [including Aurora-A, Aurora-B, Bub1B, non-SMC condensin I complex, subunit H (BRN1), Condensin, CAP-G, CAP-G/G2, CAP-H/H2, CAP-D2/D3, CAP-E, TOP2, Cyclin A, Cyclin B, CDK1, Histone H1 and inner centromere protein (INCENP)] were downregulated after treatment with the aromatase inhibitor. These results appear to be in agreement with the strong anti-proliferative effects of aromatase inhibitors in both animal and clinical studies. A second comparison was with an
in vitro study in which estrogen was removed from MCF-7 cells in culture. Decreases in genes related to the E2F1 transcription factor were observed. In our study, 13 modulated genes exhibited E2F-1 binding sites in their promoter regions, and 7 genes contained both ER binding and E2F binding sites. We were able to confirm modulation of the cell cycle related and E2F-related genes in a large independent set of human samples treated with anastrozole. The results on RNA changes for Bub 1B, Cyclin A and CDK-1 were verified by employing IHC analysis. In summary, gene changes observed in the rat closely paralleled gene changes associated with aromatase treatment and estrogen withdrawal in humans.

P3-10-03
Bardoxolone (5MeCDDO) Inhibits Cancer Initiation but Promotes Progression in Rodent Models of Breast Cancer. What Does It Mean for the Antioxidant Response Element (ARE) as a Primary Prevention Target?
Luber RA, Townsend R, Vedell P, Steele VE, Grubbs CJ. National Cancer Institute, Bethesda, MD; Washington University School of Medicine, Saint Louis, MO; Medical College of Wisconsin, Milwaukee, WI; University of Alabama at Birmingham, Birmingham, AL

The preventive efficacies of the triterprenoid 5MeCDDO were examined in three preclinical models of breast cancer. We initially evaluated 5MeCDDO in an ER+ mammary model in which female Sprague-Dawley rats were administered MNU, i.v., at 50 days of age. 5MeCDDO (27 ppm) was administered in the diet beginning 5 days after MNU, and continuing for the duration of the study. Doses >50 ppm were toxic. 5MeCDDO failed to decrease tumor latency or multiplicity and, in fact, increased the size of the cancers which did develop. This concentration of 5MeCDDO greatly increased liver to body weight ratios. We also examined the preventive efficacy of 5MeCDDO (54 and 27 mg/kg diet) in a transgenic model (MMTV-Neu/p53KO) of ER mammary cancer. In this model, animals develop cancers which overexpress Neu and fail to have a mutation in the transmembrane domain of Neu. Similarly to results in the MNU model, 5 MeCDDO did not alter either tumor latency or multiplicity. The effect of 5MeCDDO was further evaluated in a third model in which ER+ tumors were induced by the procarcinogen dimethylbenzanthracene (DMBA). DMBA was administered by gavage to female Sprague-Dawley rats at 50 days of age. In this model (which examines the ability of an agent to alter the activation of the carcinogen) the preventive agent was administered beginning 7 days prior to DMBA and was continued until 7 days post DMBA. 5,6 Benzoflavone (500 ppm), a positive control, decreased tumor multiplicity >90%, while 5MeCDDO (27 or 2.7 ppm) decreased multiplicity 65 and 35%, respectively. The efficacy observed in this model is in agreement with the ability of the agent to stimulate the ARE, and to induce a variety of ARE related genes (e.g., GST Pi, quinone reductase, etc.). Thus, as expected, high induction of the ARE was associated with a decrease in DMBA-induced cancer initiation, but not a decrease in the progression stage of mammary cancer development in three cancer models. This latter finding brings into question whether merely measuring induction of the ARE (e.g., quinone reductase) is sufficient to imply the general preventive efficacy of a given agent or mixture. Supported by NCI contract number HHSN261200433001C.

P3-10-04
Halting Early Breast Cancer Progression with Omega-3 Ethyl Esters: Altering Tumor Microenvironment in 21T Series Cell Lines.
Chen CI, Rhodes ME, Fabian C, Hursting SD, deGraffenried LA. University of Texas at Austin, Austin, TX; University of Kansas Cancer Center, Kansas City, KS

Observable infiltration of immune cells in stages of early breast cancer progression mirrors two recently conceptualized factors of carcinogenesis: tumor microenvironment and tumor-promoting inflammation.

Clinical studies using immunohistochemical staining for CD45 in patient-derived breast tissue demonstrate the involvement of leukocytes in breast cancer development, but the lack of an appropriate in vitro model has hindered molecular studies thus far. However, the 21T series cell lines effectively mimic breast cancer progression. This cell line series was obtained from the same breast cancer patient and consists of three cell lines representing atypical hyperplasia (21PT), ductal carcinoma in situ (21NT) and invasive carcinoma (21MT-1). This unique cell line series is used in our molecular study of omega-3 ethyl esters as breast cancer preventive agents. Preliminary invasion assays have described varying degrees of monocyte recruitment according to stages of progression. 21PT cells when plated on the bottom chamber failed to mobilize monocytes as effectively as 21NT or 21MT-1 and the addition of omega-3 ethyl esters suppressed the baseline invasion capacity of monocytes by 30%. Epidemiologic studies suggest that omega-3 fatty acids can act as potent cancer preventive agents and are possibly more potent when used in an ethyl ester form. Based on this, we hypothesize that omega-3 ethyl esters may prohibit early breast cancer progression through inhibiting immune cell infiltration and disrupting the formation of a tumor-promoting inflammatory and oxidative microenvironment. Phenotypic studies aim to interrogate the effect of omega-3 ethyl esters on tumor microenvironment including chemokine secretion and MMP production whereas molecular experiments probe into the importance of the Wnt pathway as a key player in the transition from ADH to DCIS. This study will demonstrate how omega-3 ethyl esters may function as breast cancer preventive agents as well as provides a model of study to test the efficacy of cancer preventive agents in vitro.

P3-11-01
Effects of Exemestane Therapy on the Lipid Profile of Postmenopausal Women with an Elevated Risk of Developing Invasive Breast Cancer.
Gatti M, Venzon D, Zujewski J, Korde L, Isaacs C, Cohen P, Warren R, Gallagher A, Eng-Wong J. Georgetown University Hospital, Washington, DC; National Cancer Institute, National Institutes of Health, Bethesda, MD; University of Washington, Seattle, WA; Lombardi Comprehensive Cancer Center, Georgetown University Hospital, Washington, DC

Background: Aromatase inhibitors are effective for breast cancer prevention in postmenopausal women. In the recent MAP.3 study, exemestane significantly reduced invasive breast cancer in postmenopausal women with an elevated risk of developing breast cancer. At 35 months follow up there was no increase risk in cardiovascular events in this study; however, the effects of exemestane use on lipid profiles and cardiovascular health are still unclear.

Methods: We conducted a single-arm phase II trial of exemestane in women at increased risk for breast cancer and examined the impact of exemestane on lipid profiles. Postmenopausal women at high risk
for invasive breast cancer (e.g., Gail Model risk ≥ 1.7, a history of lobular neoplasia, atypical ductal hyperplasia, DCIS, or stage I/II breast cancer, or BRCA1/2 mutation) were given exemestane (25 mg orally daily) for 2 years. Fasting serum total cholesterol, HDL, LDL, triglycerides, and homocysteine were collected at baseline, 3, 12, and 24 months after initiation of exemestane therapy. Apolipoprotein A and B were collected at baseline, 3 and 12 months. Wilcoxon sign ranked test was used to analyze if changes from baseline values differed from zero. The Hochberg p-value adjustment was used to account for multiple hypothesis tests. Results: Of the 42 women enrolled in the study, 6 dropped out prior to completing 1 year and 1 dropped out prior to completing 2 years of exemestane therapy. Thirty-one women have completed 2 years of exemestane therapy and the remaining 4 are expected to complete 2 years of therapy by January 2012. On average, participants were 58.5 years old, mostly Caucasian (n = 37; 84.1%), and had a BMI of 29.2 kg/m2. A majority (n = 19) of participants were on lipid-lowering medications (14 were taking a statin) or taking fish oil supplements (n = 5) prior to starting on the trial and 1 was started on a statin approximately 10 months after starting the trial. There were no significant differences in mean lipid values for each of the 4 assessment points or in the mean change from baseline at 3, 12, and 24 months between patients who were taking lipid-lowering medications and those who were not. In unadjusted analyses, change in HDL from baseline was significantly different from zero and decreased from baseline at 3, 12 and 24 months (-8.0 mg/dL, -8.5 mg/dL, and -9.9 mg/dL; All p-values ≤ .001 before and after applying the Hochberg adjustment). Total cholesterol also significantly decreased from baseline at 3 months (-13.6 mg/dL, p = .002) but was no longer significant at 12 and 24 months (-9.6 mg/dL and -11.4 mg/dL, respectively; p-values = .07). The rest of the lipid panel did not significantly change during follow-up.

Discussion: In agreement with previous studies, we found that exemestane causes a significant decrease in HDL and total cholesterol, while leaving the rest of the lipid panel unchanged. Prior studies excluded patients on lipid-lowering medication; half of our participants were taking lipid-lowering medication. It is notable that both women off and on lipid-lowering medication had decreases in HDL. Additional studies are needed to elucidate long-term cardiovascular outcomes in this high risk but otherwise healthy population. 

P3-11-02 Changing Paradigms for Breast Cancer Chemoprevention? 
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The SERMs tamoxifen and raloxifene are approved for breast cancer risk reduction but their acceptance in clinical practice has been poor largely related to rare but potentially life threatening side effects which requires identification of populations at higher breast cancer risk and careful risk benefit discussion (Visvanathan 2009). Recent findings from randomized placebo-controlled clinical trials suggest potential alternative approaches. In the MAP3 placebo-controlled trial including 4,650 postmenopausal women, exemestane decreased breast cancer incidence by 65% (P=0.002) (Goss 2011) with no significant difference between groups for fractures, CVD events, other cancers or treatment-related deaths. While the median Gail 5-yr risk score of participants was 2.3, eligibility factors included age alone (≥ 60 years) regardless of calculated breast cancer risk and accounted for 49% of entries. With extended follow-up from the Women’s Health Initiative (WHI) randomized, placebo-controlled trial evaluating estrogen alone in 10,739 postmenopausal women with prior hysterectomy, a statistically significant decrease in breast cancer incidence of 23% was seen with estrogen use (P=0.02) (LaCroix 2011). Younger postmenopausal women (age 50-59 years at entry) randomized to estrogen alone had lower risk of myocardial infarction (HR 0.54, 95% CI 0.34-0.86) and death (HR 0.73, 95% CI 0.53-1.00). These findings contrast to those in the WHI trial evaluating combined estrogen plus progestin in 16,608 postmenopausal women with an intact uterus where breast cancers were increased 25% (P=0.004) as was breast cancer mortality (increased by 96%, P=0.049) (Chlebowski 2010). The apparently paradoxical findings that in postmenopausal women estrogen addition, as conjugated equine estrogen, lowers breast cancer incidence and estrogen reduction, by aromatase inhibitor use, also lowers breast cancer incidence are consistent with preclinical studies indicating condition dependent change in estrogen influence on mammary cancers (Jordan 2011). Major eligibility for the WHI trials included anticipated 3-yr survival regardless of breast cancer or CVD risk. The findings from the MAP3 and WHI trials highlight the complex relationships between estrogen and breast cancer and suggest potential strategies for chronic disease risk reduction in select populations of postmenopausal women which may not require formal breast cancer risk assessment.


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Background: D-limonene (DL) is a highly lipophilic monoterpene found naturally in citrus that has been demonstrated in preclinical studies to have anticancer properties. Early phase clinical trials support the investigation of DL in the chemoprevention of breast cancer. We sought to evaluate whether DL and its presumed active metabolite perillic acid (PA) would distribute extensively to the breast tissue and reach an effective drug concentration. We hypothesized that the mechanism of DL is related to the induction of apoptosis in cancer cells.

Materials and Methods: We enrolled 40 patients with newly diagnosed Stage 0-2 breast cancer to take 2 grams daily of oral DL for 2-6 weeks prior to planned surgical intervention. Blood was drawn pre/post intervention to assess for toxicity and plasma concentration of DL and PA. Adverse effects related or possibly related to the study drug were noted. A small piece of breast tissue adjacent to the tumor mass was used to measure drug concentration for each patient. DL and PA levels in breast tissue were analyzed by gas chromatography and liquid chromatography, respectively, in tandem with mass spectrometry. Analysis of variance testing was used to determine if DL or PA preferentially concentrated in the breast tissue compared to plasma. We tested for a possible modulation of the biomarkers estrogen receptor (ER), progesterone receptor, HER2, Ki67 or grade pre/post treatment by comparing results from the core biopsy to the surgical pathology and applying pairwise Student’s T tests. The caspase 3 and the annexin V assays were performed by platting 10,000 cells
per well for the cell lines MCF7, MDA-231, BT474, and T47D and separately administering DL and PA acid in serial dilutions in their treatment concentration ranges in triplicate and read by microplate. Results: DL was found to preferentially concentrate in breast tissue versus plasma (tissue/plasma concentration ratio (TPCR) of 1297, p<0.001) while PA did not concentrate (TPCR of 1.4, p=0.9). 20 patients (50%) reported Grade 1 irritation, which was the most common adverse effect. A slight decrease in white count from a mean of 7.1 to 6.6 (p=0.03) and a slight increase in ALT from a mean of 22.5 to 26.9 (p=0.03) were noted. No other statistically significant changes in laboratory values related to serum complete blood count, renal, hepatic or other studies were noted. No change in tissue biomarkers were noted post-treatment. DL produced a dose dependent increase in the apoptotic markers cleaved caspase 3 and annexin V for the ER positive cell lines MCF7, BT474, and T47D; however, no such response was noted for the triple negative cell line MDA-231. A PA dose response trend was noted with the annexin V but not the caspase 3 assay for each cell line. Discussion: D-limonene preferentially concentrates in the breast tissue and is a candidate chemopreventive agent based on its favorable side effect profile. PA does not readily concentrate in the breast when administered as oral DL. Our correlative studies establish that DL induces apoptosis in ER positive breast cancer cell lines; no definite relationship between PA and apoptosis was found. Further clinical trials of DL are necessary to establish its potential role as a chemopreventive agent in breast cancer.

P3-11-04
A Randomized Phase II Biomarker Study of Atorvastatin in Premenopausal Women at Increased Risk for Breast Cancer.
Wood ME, Kingsley F, Ellerton JA, Atkins JN, Grabs SS, Mass HB, Garber JE. University of Vermont, Burlington, VT; Cancer Consultants, Las Vegas, NV; Southeastern Medical Center, Winston-Salem, NC; Medical Oncology Hematology Consultants, Newark, DE; University of North Carolina Lineberger Comprehensive Cancer Center, Chapel Hill, NC; Dana-Farber Cancer Institute, Boston, MA.

Statins have been shown in epidemiological and laboratory studies to have breast cancer risk reduction properties. We evaluated the effect of atorvastatin on mammographic density (MD) and other breast cancer biomarkers in a small randomized placebo controlled trial.

Methods: Premenopausal women at increased risk for breast cancer (due to family history, BRCA positivity, prior biopsy history or history of chemoradiotherapy for Hodgkins disease) enrolled after signing informed consent and received either 40 mg of atorvastatin or placebo daily for 1 year. Biomarker assessment was performed prior to initiation and prior to completion of study medication. MD was determined using both Breast Imaging Reporting and Data System (BI-RADS) and the Visual Analogue Scale (VAS).

Results: 63 women were enrolled between 7/05 – 8/10 in this multi-institutional trial. 44 have completed study medication and 3 remain on study. 25% have withdrawn; 17% of them for toxicity/side effects. Of those completing the study; mean age was 47 (range 35-50), 96% Caucasian, mean BMI 26.8. 66% had a strong family history (2% were BRCA+); 27.7% had ADH/LCIS. We present analysis of MD in the first 37 women completing study drug. The two treatment groups were well balance for age, BMI, risk factors and baseline density. Mean density by VAS was 31.6% at study entry and 32.4% at end of treatment. There was no different in change in density over time between atorvastatin and placebo using either BIRAD or VAS; controlling for BMI did not change results. Futility analysis demonstrates low probability of a significant difference between treatment groups and the study was closed.

Conclusions: In this multi-institutional randomized prospective clinical trial of premenopausal women at increased risk for breast cancer we have failed to demonstrate a significant effect of atorvastatin on mammographic density. There are several possible reasons for this relating to: the study population, method of density determination and/or biomarker analyzed. While the primary aim of this study (MD) was not met we have shown that biomarker studies can be done in a multi-institutional setting. Further biomarker evaluation may prove informative.

Funding: This study is sponsored by grants from Breast Cancer Research Foundation and Cancer and Leukemia Group B to M. Wood.

P3-11-05
An Efficient Resource To Accelerate Research into the Cause and Prevention of Breast Cancer: The Love/Avon Army of Women. 

Love SM, Dr. Susan Love Research Foundation, Santa Monica, CA

Background: It is well established that more research into the cause and prevention of breast cancer is needed. While many studies are done in cell lines and laboratory animals, translation of findings to women often falters due to perceived difficulty in recruiting women for research. The Dr. Susan Love Research Foundation received a grant from the Avon Foundation to form the Love/Avon Army of Women (AOW); an on-line recruitment resource launched in 2008, designed to partner one million women with the research community in an effort to accelerate breast cancer research.

Methods: Researchers submit a proposal to the AOW Scientific Advisory Committee. If a study is accepted, a mass e-mail describing the study procedures and inclusion/exclusion criteria is sent to the entire AOW database. Women sign up at www.armyofwomen.org to join and receive AOW e-mails about breast cancer research studies. Women self select based on interest and study criteria, and undergo a secondary on-line screening before contact information is passed on to the researcher for the enrollment process.

Results: Over 356,000 women have signed up, including survivors and healthy women, ranging from ages 18 to 100, representing all 50 US states and 49 countries. To date, the AOW has recruited for 48 studies, both regional and national, that vary from biomarker and/or circadian rhythm research to psycho-social and quality of life studies. With over 54,000 AOW members having participated in the research process, this method of recruitment has been found to be effective and efficient. The diversity of the AOW members has proved beneficial for many studies, such as those needing to enroll racial/ethnic minorities, women of varying sexual orientations, or young survivors. Nine studies were closed to enrollment within the first 48 hours after accruing all subjects while others were very quickly over-enrolled by 5-10%. A urine biomarker study in search of healthy women and breast cancer survivors closed within eight hours, after 2,076 women signed up, representing a 58% over-enrollment. This overwhelming response has led many researchers to seek additional funding to increase their statistical significance and accommodate all eligible subjects.

Conclusions: The AOW has proved to be a successful resource for scientists to accelerate accrual, expand the number and diversity of their subject population and to obtain exactly the type of specimens they need when they need it. This partnership between women and scientists has revolutionized research and accelerated efforts to eradicate breast cancer.
P3-11-06
A Pilot Study of RPFNA in Overweight and Obese Postmenopausal Women.
Korde LA, Grieco VS, Inamaya I, Kumai C, Mason C, Duggan CR, Wang C-Y, McTiernan A. University of Washington, Seattle, WA; Fred Hutchinson Cancer Research Center, Seattle, WA

Background: Random periareolar fine-needle aspiration (RPFNA) is a research-based procedure used to obtain breast epithelial cells and fluid. Data suggest that atypia in epithelial cells from RPFNA specimens is associated with an increased risk of breast cancer, and changes in the degree of atypia may be a viable biomarker for response to chemopreventive interventions.

Methods: We performed RPFNA on 37 overweight or obese (body mass index (BMI) ≥ 25.0 kg/m²) postmenopausal (50-75 y) women enrolled in a randomized clinical trial of Vitamin D vs placebo in combination with a lifestyle (diet + exercise)-based weight loss program. Eligibility criteria included serum vitamin D concentrations 10-32 ng/mL. The procedure was performed by two trained clinicians prior to randomization to assess baseline cell counts and degree of atypia. Two areas in the breast (10:00 and 2:00 positions) were infiltrated with lidocaine, and 8 to 10 aspirations of cellular material were performed using 1.5 inch, 21-gauge needles. The aspirated fluid was placed in 9 cc of Cytolyt Thinprep (Hologic Inc.) with 1 cc of 1% neutral buffered formalin and slides were prepared according to the University of Washington Department of Pathology non-gyn protocol. Results: Thirty-seven women were included in this analysis. The mean age and BMI of study participants was 59.3 years and 32.5 kg/m², respectively. The mean 5-year Gail risk score for study participants was 1.96%; 52% of women had a 5-year Gail risk ≥ 1.66%. Eight participants (21.6%) had >50 cells present on cytology examination. One additional participant had 10-50 cells, and one had <10 cells. No specimens showed cellular atypia. The remainder of study participants had only blood or fibroadipose tissue on cytology specimens. Women with epithelial cells present were younger (mean age 55.0 vs. 60.2 years, p=0.05). There were no significant differences in BMI or Gail risk score between those with and without epithelial cells. Conclusions: RPFNA in our sample of overweight/obese postmenopausal women did not yield epithelial cells in a majority of participants. Younger age was associated with a greater cell count. We are planning further studies to determine whether other markers in RPFNA specimens can be used to assess breast tissue changes in response to chemopreventive interventions.

P3-11-07
Mammary Gland Biopsy To Examine Surrogate Endpoint Biomarkers of Preventive Agent Efficacy.
Heckman-Stoddard B, Lubet RA, Bode AM, Grubbs CJ. National Cancer Institute, Bethesda, MD; University of Minnesota, Austin, MN; University of AL at Birmingham, Birmingham, AL

Phase II breast cancer prevention trials examine agent activity by sampling and imaging the breast through core needle biopsy or fine needle aspiration, or imaging (e.g., mammography). The biomarker endpoints in normal or at risk breast tissue employed with biopsies or fine needle aspirates (e.g., Ki67 or apoptosis) have often not been formally validated relative to a tumor endpoint. In this study, we have attempted to examine these endpoints in a preclinical model for which we have final tumor data. In the methylnitrosourea (MNU)-induced rat mammary cancer model, estrogen receptor positive cancers develop that are histologically similar to a large majority of human tumors. Here we present a protocol for correlating surrogate endpoint biomarkers and agent efficacy in the same animal. Biopsy samples are taken of the normal mammary gland after MNU treatment, and a week later the animals are started on the preventive agent. After 2 weeks, a second biopsy is taken and the animals are followed for tumor development. To test the protocol, we examined surrogate endpoints of tamoxifen efficacy at human equivalent dosages (5mg/d and 20mg/d). Both doses of tamoxifen prevented the development of tumors in the MNU-treated rats. The pre/post biopsy analysis revealed a statistically significant reduction in Ki-67 consistent with the clinical trial data. Additional biomarkers of apoptosis and the chromosome condensation pathway will be presented.

P3-12-01
Postma EL, Verkooijen HM, van Esser SE, Hobbelen MG, van der Schelling GP, Koellemij R, Witkamp AJ, Contant CM, van Diet PJ, Borel Rinkes IH, van den Bosch MA, Mali WP, van Hillegersberg R. UMC Utrecht, Utrecht, Netherlands; St. Antonius ziekenhuis, Nieuwegein, Netherlands; Amphia Hospital, Breda, Netherlands; Maasstad Hospital, Rotterdam, Netherlands

Background
For the management of non-palpable breast cancer, accurate pre-operative localization is essential to achieve complete resection with acceptable cosmetic results. ROLL takes advantage of the intratumorally injected radiotracer, that is already used for the sentinel node procedure, to localize the primary tumor during surgery. In a multicenter randomized controlled trial, we determined if ROLL is superior to the standard of care (i.e. wire guided localization, WGL) for preoperative tumor localization.

Methods
Women (>18 yrs.) with histologically proven non-palpable breast cancer and eligible for breast conserving treatment (BCT) with sentinel node procedure were randomized to ROLL or WGL. Patients allocated to ROLL received an intra-tumoral dose of 120 Mbq Technetium99 nanocolloid. Guided by a gamma detection probe, the surgeon excised the primary tumor and the sentinel node(s). In the WGL group, patients received a similar intra-tumoral or periaureolar dose of technetium in order to allow sentinel node biopsy. Ultrasound or mammography guided insertion of a hooked wire provided surgical guidance for excision of the primary tumor. Primary outcome measure was the proportion of complete tumor excisions (i.e. with negative margins). Furthermore, the proportion of patients requiring re-excision was assessed. Data were analyzed according to intention to treat analysis.

Results
Three hundred and fourteen patients with 316 invasive breast cancers were enrolled. There were no significant differences in proportion of complete tumor removal with free margins and re-excision rate; complete tumor removal with negative margins was seen in 87.7% of patients in the ROLL group versus 85.5% (p=0.97) of patients in the WGL group. Re-excision was required in 11.7% of patients in the ROLL group versus 9.2% (p=0.47) in the WGL group. Incorrect pre-operative localization (i.e. failure to identify the correct localization of the lesion) occurred in 5 (3%) patients in the ROLL group versus 0 patients in the WGL group (p=0.029). Differences in the volume of the excised specimen, duration of the procedure, success rate of the sentinel node procedure, surgeons’ preferences and patients’ pain perception are currently being analyzed.

Conclusion
With this multicenter randomized controlled comparison, the first of its kind in patients with histologically proven breast cancer, we show that ROLL is not superior to WGL in terms of complete tumor excision and re-excision rates. Furthermore incorrect lesion localization occurred more frequently when applying ROLL. Data on long term (6 months) cosmetic results and quality of life (6 weeks, 3 months, 6 months) are awaited.

**P3-12-02**

*Intra-Operative Margin Assessment of Diffuse Disease with MarginProbe™ as an Adjunct to Standard of Care, Results from a Randomized Prospective Multi Center Study.*

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**Background**

The ability to obtain negative margins with a single surgical procedure remains a challenge, particularly in patients with diffuse disease such as DCIS and lobular pathology. A novel device (MarginProbe, Dune Medical Devices, Inc.) is intended to provide surgeons with real time, intraoperative detection of cancerous tissues at the margins of excised specimens. A study was performed to determine if there was a device-associated improvement in complete surgical resection (CSR) and therefore a decrease in the rate of patients requiring re-excision with these disease types. The current analysis stratified the data based on tumor type with a special focus on DCIS patients, patient with a DCIS component, and lobular patients.

**Methods**

All 596 patients underwent breast conservation, with image-guided localization, and were randomized in a prospective, international, multicenter (n=21) study. Randomization occurred in the operating room, following standard lumpectomy procedure, including palpation followed by additional cavity resections as indicated. In the device arm, MarginProbe was used on each specimen margin and device positive readings required additional resections of the cavity. Pathologists were blinded to study arm. Re-excision criteria were not dictated by the protocol.

A primary endpoint of this study was CSR, defined as the correct intraoperative identification and resection (if not skin or fascia) of all positive margins on the main lumpectomy specimen. Positive lumpectomy specimens were defined as those having at least one margin having cancer ≤1mm from the surface. Successful CSR results in reduced positive margin rate after lumpectomy.

**Results**

Results are presented in Table 1. The improvement in CSR was significant for all diagnosis types. The decrease in candidates for re-excision due to failed CSR was significant for all DCIS and mixed tumor types.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Group</th>
<th>Total Patients</th>
<th>Patients having Successful CSR (%N)</th>
<th>p-value</th>
<th>Candidates for re-excision (%N)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCIS</td>
<td>Device</td>
<td>83</td>
<td>69% (57/83)</td>
<td>0.0001</td>
<td>11% (9/83)</td>
<td>0.0005</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>79</td>
<td>77% (61/79)</td>
<td></td>
<td>16% (12/79)</td>
<td></td>
</tr>
<tr>
<td>Mixed (DCIS + Invasive)</td>
<td>Device</td>
<td>155</td>
<td>73% (114/155)</td>
<td>0.0006</td>
<td>16% (25/155)</td>
<td>0.0004</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>179</td>
<td>77% (138/179)</td>
<td></td>
<td>16% (30/179)</td>
<td></td>
</tr>
<tr>
<td>Invasive Lobular</td>
<td>Device</td>
<td>33</td>
<td>87% (29/33)</td>
<td>0.047</td>
<td>10% (3/33)</td>
<td>0.52</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>40</td>
<td>85% (34/40)</td>
<td></td>
<td>12% (5/40)</td>
<td></td>
</tr>
</tbody>
</table>

**Conclusions**

Use of the device resulted in significant improvement in CSR and therefore a significant decrease in the need for reexcisions. Further studies should be conducted to evaluate the use of the device for additional patient cohorts, such as patients receiving neoadjuvant treatment and patients who have undergone prior breast surgery.

**P3-12-03**

*A Prognostic Index of Ipsilateral Breast Tumor Recurrence in Patients Treated with Breast-Conserving Surgery after Preoperative Chemotherapy: Validation of M.D. Anderson Prognostic Index.*

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**Background**

Preoperative chemotherapy (PCT) is widely used to increase the possibility of breast-conserving treatment (BCT). However, the appropriate indication for BCT after PCT is controversial, because the rates of ipsilateral breast tumor recurrence (IBTR) may be higher than those reported for BCT when surgery is used first. We performed a multicenter retrospective study to evaluate factors that were associated with IBTR in patients with BCT after PCT, and validated M. D. Anderson Prognostic Index (MDAPI) (Cancer 2005;103:689-95) using our data set.

Patients and Methods: From eight Japanese hospitals, data were extracted on a total of 381 patients with invasive breast cancer (BC) who were treated with ≥2 cycles of PCT followed by breast-conserving surgery and irradiation. The rates of IBTR were evaluated by MDAPI including clinical N2 or N3 disease, pathologic residual tumor >2 cm, multifocal pattern of residual disease, and lymphovascular space invasion in the specimen. Kaplan-Meier method was used to estimate cumulative recurrence rates. Log-rank test and Cox’s proportional hazard model were used for statistical analyses.

Results: Median age at diagnosis of the primary tumor was 48 years; median size of the primary tumor at diagnosis was 4.0 cm. One hundred and forty-six patients received postoperative chemotherapy and 211 received postoperative endocrine therapy. At a median follow-up period of 50 months, 18 of 381 patients developed IBTR, which resulted in 5-year IBTR-free rate of 94.1%. Univariate analyses revealed that estrogen receptor (ER) status both before and after PCT (positive vs. negative), pathological nodal status after PCT (≥4 vs. 0-3 positive nodes), and pathologically residual invasive tumor (≥1.8 vs. ≤1.7 cm) were significantly associated with IBTR (all P < 0.05). Pathological margin status did not affect IBTR rate (P=0.88).

Multivariate analysis revealed that significant independent predictors of IBTR included ER status after PCT (Hazard Ratio [HR], 0.10; P<0.01), size of residual invasive tumor (HR, 5.29; P=0.05), and pathological nodal status after PCT (HR, 3.59; P=0.02). The rates of IBTR of patients with MDAPI 0-3 were 1.3%, 2.9%, 16.0%, and 3.6%, respectively. Based on the data of our multivariate analysis, ER status after PCT (ER positive:0 and ER negative: 1 was added to MDAPI. Total scores of the prognostic index including MDAPI and ER status after PCT ranged between 0 and 5. The rates of IBTR correlated well with this prognostic index. The 5-year IBTR-free survival rates were 0% for 23 patients in score 0, 3.4% for 89 in score 1, 3.9% for 51 in score 2, 21.2% for 33 in score 3, and 16.7% for 6 in score 4 (P<0.01).

Conclusion: Our prognostic index (MDAPI plus ER status) would be useful for clinical decision making according to surgical procedures after PCT. BCT is an appropriate treatment option for patients with the low prognostic index (0 to 2). The high risk population with the high prognostic index (3 to 5) may benefit from mastectomy.
P3-12-04
Involved Anterior Margins after Breast Conserving Surgery: Is Re-Excision Required?
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Background: Complete tumour excision in breast conserving surgery (BCS) is critical for successful treatment; involved circumferential resection margins are associated with increased disease recurrence. However, the importance of an involved anterior margin is less clear. The purpose of this study was to audit an aggressive approach to involved anterior margins and hence assess whether anterior margin re-excision yields clinical benefit.

Material and Methods: A retrospective case note and pathology review was performed for all patients who underwent BCS between 2006 and 2010 through a single cancer centre. An involved margin was defined as <1mm clearance of invasive or in situ breast cancer. Results: 1667 patients underwent BCS for invasive and/or in-situ disease, of whom 114 (6.8%) underwent re-excursion, most commonly for mixed invasive and in-situ pathology. The annual re-excision rate rose significantly (p=0.001), with no change in whole tumour diameter, specimen weight or specimen volume. A total of 170 involved margins were identified: most commonly the anterior margin (59 margins, 30.6%) followed by the posterior (39 22.9%) or inferior (31, 18.3%) margin. Patients with anterior margin involvement were more likely to have grade 3 invasive disease (p=0.0323) but less likely to have residual disease found at re-excision (2/49 vs. 32/101 margins, p=0.0033); there were no differences when in-situ characteristics were compared.

Conclusions: Re-excision of involved anterior margins rarely excises residual disease and may be unnecessary. Multidisciplinary teams should consider whether further therapy for an involved anterior margin is required on a patient by patient basis.

P3-12-05
Breast Cancer Recurrence: 2nd Conservative Treatment Versus Mastectomy.
Michel V, Houvenaeghel G, Bannier M, Tallet A, Minsat M, Lambaudie E, Salem N, Buttarelli M, Resbeut M. Paoli Calmettes Cancer Institute, Marseille, France

Objectives: Mastectomy (Mt) is considered standard treatment for isolated local recurrence (LR) of breast carcinoma. The aim of our study was to evaluate a second conservative treatment (defined as lumpectomy followed by interstitial brachytherapy (LpIB)) and to determine if it compares favorably with the current standard treatment.

Materials and methods: Between January 1981 and December 2009, 348 patients were treated to the Paoli Calmettes Institute (IPC) for an isolated LR: 232 (66.7 %) underwent Mt, 62 (17.8 %) received a second radio-surgical conservative treatment (LpIB) and 54 (15.5 %) a 2nd single surgical treatment (Lp).

We classified each population according to the well known prognosis factors. Then, populations Mt and LpIB were matched taking into account these criteria to compare the overall survival (OS), metastasis free survival (MFS) and 2nd local recurrence free survival according to the treatment delivered.

Results: On 348 reviewed patients, with a median follow-up of 73.3 months, 65 patients died (42/232 Mt, 8/62 TecCur, 15/54 Tec) and 100 presented metastasis (64/232 Mt, 15/62 TecCur, 21/54 Tec). There was no difference in MFS for the 2 groups, LpIB and Mt (80 % at 5 years) and the OS was non significantly better in the group LpIB compared to the group Mt (90 % and 82 % at 5 years respectively, p=0.28), whereas in the LpIB group 17 % and 30 % presented a relapse at 5 years and 10 years respectively. They subsequently underwent a salvage mastectomy.

Worse results were obtained with lumpectomy alone (OS = 72 % and MFS = 68 % at 5 years) compared with 2 other option treatments.

Conclusion: A second conservative treatment for breast cancer recurrence, i.e. lumpectomy and interstitial brachytherapy, is possible for selected patients, without any negative impact on overall survival, nor metastasis free survival.

Keywords: Local recurrence, interstitial brachytherapy, lumpectomy, conservative treatment, mastectomy, overall survival, metastasis free survival, breast carcinoma.

P3-13-01
Boost Radiation Therapy Not of Value in Reducing IBTR of Invasive or Noninvasive Breast Cancers for Patients with DCIS:
Results from the NSABP B-24 Trial.
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Background: Whole breast irradiation therapy following lumpectomy for invasive breast cancer (IBC) or noninvasive breast cancer (DCIS) significantly reduces the risk of local recurrence. Boost radiation therapy to the tumor bed has been proven to additionally lower the risk of recurrence for IBC. The benefit of boost therapy in patients with DCIS is less certain. We carried out a review of the NSABP B-24 trial to assess the benefit of boost therapy.

Methods: After lumpectomy and radiation therapy, 1804 women with DCIS were randomly assigned to placebo (902) or tamoxifen (902). Whole breast irradiation therapy (50 Gy) was mandatory. Boost radiation therapy was optional, and boost status was known for 1,569 patients. Of these, 1392 patients (86.9%) were identified as having all data sufficient for multivariate analysis. Of these, 613 received boost therapy ranging from 1 Gy to 20 Gy, with 81.5% receiving 10 Gy. Mean time of follow-up was 161 months.

Results: Patients who received boost radiation therapy were more likely to be younger (p<0.04), have positive margins (p=0.007), and be more likely to have comedo necrosis (p=0.03). Multivariate analysis identified only treatment (tamoxifen vs placebo) (HR=0.74, 95% CI=0.57-0.98, p=0.034), age (≥ 50 vs < 50) (HR=0.47, 95% CI=0.36-0.61, p<0.0001), and margin status (positive vs negative) (HR=1.79, 95% CI=1.31-2.43, p<0.001) as significant predictors for ipsilateral breast tumor recurrence (IBTR). Boost had no significant effect on IBTR (HR=0.87, 95% CI=0.66-1.15, p=0.33). The lack of boost effect applied to both invasive (HR=0.86, 95% CI=0.58-1.27, p=0.44) and noninvasive IBTR (HR=0.89, 95% CI=0.60-1.33, p=0.56). No interaction was seen between boost and treatment, age, margin status, or comedo necrosis.

Conclusion: In NSABP B-24, the addition of boost radiation therapy was not found to be of value in reducing IBTR of invasive or noninvasive breast cancers for patients with DCIS.

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P3-13-02
The Impact of Lymph Node Status on Clinical Outcomes Following Accelerated Partial Breast Irradiation.
Shah C, Wilkinson JB, Wallace M, Vicini F. William Beaumont Hospital, Royal Oak, MI

Background: Limited data exists on outcomes following accelerated partial breast irradiation in node-positive breast cancer patients. The purpose of this analysis was to compare clinical outcomes following accelerated partial breast irradiation (APBI) between node-negative and node-positive early stage breast cancer patients and to identify if nodal positivity leads to increased rates of local or axillary failure. Materials and Methods: 510 patients with early stage breast cancer received accelerated partial breast irradiation (APBI) as part of their breast conservation therapy between April 1993 and November, 2010. Of these, 39 were lymph node positive with 10 patients having N1mi disease (median size of mets= 0.82 mm) and 29 patients having N1 disease (61.5% had one node positive [median size of mets= 2.5 mm], 30.8% had 2 nodes positive [median size of mets= 8.0 mm], and 7.7% had 3 nodes positive [median size of mets= 20 mm]). Patient, clinical, and pathologic factors were analyzed and compared for the node-negative and node-positive cohorts including age, tumor size, receptor status, margin status, adjuvant hormonal therapy, adjuvant chemotherapy, and length of follow-up. Clinical outcomes were analyzed including local recurrence (LR), regional recurrence (RR), axillary recurrence (AR), regional-nodal recurrence (RR), distant metastases (DM), disease-free survival (DFS), cause-specific survival (CSS), and overall survival (OS).

Results: Node-positive patients were younger (p=0.04), had larger tumors (p<0.001), and were more likely to receive chemotherapy (p<0.001). Median follow up was 5.3 years for node-negative patients and 5.9 years for node-positive patients (p=0.06). At 5 years, no differences were seen in the 5-year actuarial rates of LR (2.2% v. 2.6%, p=0.86), AR (0% v. 0%, p=0.69), DFS (90.0% v. 88.0%, p=0.79), CSS (98.0% v. 90.0%, p=0.06), or OS (91.0 v. 84.0%, p=0.65) while higher rates of RR (0% v. 6.1%, p=0.001) and DM (2.2% v. 8.9%, p=0.005) were noted in node-positive patients. A total of 10 LRs occurred in the node-negative patients and 1 LR in the node-positive patients at a median of 2.6 and 1.5 years respectively. Both RR that developed in node-positive patients were within the supraclavicular fossa at a median of 2.8 years. Univariate analysis of LR was performed and age (p=0.31), tumor size (p=0.48), ER status (p=0.13), PR status (p=0.34), T-stage (p=0.48), chemotherapy (p=0.41), APBI technique (p=0.80), and nodal status (p=0.86) were not associated with LR while there was a trend for the association of LR with close/positive margins (p=0.07), and failure to receive adjuvant hormonal therapy (p=0.06). No variables were associated with any type of AR.

Discussion: No difference was seen in the rates of local recurrence or axillary failure between node-negative and node-positive following APBI with 5-years of follow-up. Increased rates of regional failure were noted with APBI in node-positive patients due to supraclavicular failures; however, traditional whole breast irradiation techniques would not have covered these failures.

P3-13-03
Long-Term Symptoms after Radiotherapy of Supraclavicular Lymph Nodes in Breast Cancer Patients.
Lundstedt D, Gustafsson M, Steineck G, Alsadius D, Sundberg A, Wilderäng U, Holmberg E, Johansson K-A, Karlsson P. Institute of Clinical Sciences, Sahlgrenska Academy at University of Gothenburg, Gothenburg, Sweden; Institute of Clinical Sciences, Gothenburg, Sweden; Karolinska Institute, Stockholm, Sweden

Background and Purpose: Irradiation of the supraclavicular lymph nodes has historically been shown to increase the risk of brachial plexopathy with neurological problems in the upper limb. The purpose of this study was to compare long-term symptoms after modern radiotherapy (based on 3D dose planning) in breast cancer patients with or without irradiation of the supraclavicular lymph nodes.

Material and Methods: We collected information from 814 recurrence free women consecutively treated with adjuvant radiotherapy for breast cancer at the Sahlgrenska University Hospital in Gothenburg, Sweden, 1999 to 2004. The women had breast conserving surgery or mastectomy with axillary dissection or sentinel node biopsy. The breast area was irradiated to 50 Gy in 2.0 Gy fractions. Women with more than three lymph node metastases had regional radiotherapy to the supraclavicular lymph nodes delivered in 2.0 Gy fractions up to 50 Gy. Systemic treatments were given according to regional guidelines. In this study the women were classified into three groups depending on if they had axillary dissection and regional radiotherapy. The first group had both axillary dissection and regional radiotherapy, the second group had axillary dissection without regional radiotherapy, and the third group had sentinel node biopsy (i.e. no axillary dissection) without regional radiotherapy. Three to eight years after radiotherapy, the women received a questionnaire asking about paresthesia, pain and strength in the upper limb.

Results:
Among women with axillary dissection and regional radiotherapy 38/192 (19.8%) reported paresthesia in the hand compared to 68/505 (13.5%) among women with axillary dissection without regional radiotherapy; relative risk (RR) 1.47; 95% confidence interval (95% CI) 1.02 – 2.11, and compared to 9/112 (8.0%) among women with sentinel node biopsy without regional radiotherapy; RR 2.46 (95% CI 1.24-4.90). Type of breast surgery, number of examined axillary lymph nodes, and chemotherapy had no impact on the occurrence of paresthesia. Age was an effect modifier among the women with axillary dissection and regional radiotherapy; up to 49 years of age 26.8% reported paresthesia (RR 2.45; 95% CI 1.05-5.73), between 50 and 59 years of age 19.7% reported paresthesia (RR 1.81; 95% CI 1.24-4.90), and above 59 years of age 10.9% reported paresthesia (RR 1.81; 95% CI 1.02 – 3.09). Long-Term Symptoms after Radiotherapy of Supraclavicular Lymph Nodes in Breast Cancer Patients.

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P3-13-04

Baschnagel A, Shah C, Wilkinson JB, Dekhne N, Margolis J, Arthur DW, Vicini F. William Beaumont Hospital, Royal Oak, MI; VCU Massey Cancer Center, Richmond, VA

Purpose: To examine the rate of breast reconstruction failure and cosmetic outcomes following post-mastectomy radiation therapy (PMRT) with temporary tissue expander or implant in place.

Methods and Materials: Ninety-four patients with 95 primary breast malignancies underwent mastectomy and immediate tissue expander reconstruction followed by PMRT. Ninety tissue expanders and five implants were irradiated. All patients received a dose of 5,400 cGy given in 180 cGy fractions. Twenty-one patients (22%) received tangents alone and seventy-four patients (78%) were treated with tangents and a supraclavicular field via a mono-isocentric technique. Bolus was used in 91 patients (96%). Eighty-eight patients (93%) received chemotherapy and 78 patients (82%) received endocrine therapy.

Results: With a median follow up of 22.4 months, nineteen patients (20%) developed a reconstruction failure. Ten patients lost their tissue expanders with a median time to reconstruction failure of 7 months (range 3-9 months) while ten patients lost their permanent implant with a median time to loss of 19 months (range 9-31 months) following PMRT. The one and two-year actuarial rate of reconstruction failure was 11.2% and 24.9%, respectively. The most common cause of failure was infection (37%), followed by skin break down (21%), extrusion of expander or implant (16%), fibrosis/eschar (11%), trauma (11%) and pain (5%). Out of the 19 failures, eight patients were salvaged with an autologous flap reconstruction. Univariate analysis was performed to examine the association between age, chemotherapy, hormonal therapy, smoking status, hypertension, diabetes, menopause status, weight and the use of a third supraclavicular RT field on reconstruction failure. However no risk factors were found to be associated with reconstruction failure. In patients who did not have reconstruction failure, good/excellent cosmesis was observed in 79% of patients and fair/poor cosmesis was observed in 21% of patients.

Conclusions: In our series of women with a high risk of recurrence, PMRT with a tissue expander in place followed by a prosthetic implant provides good cosmesis in the majority of women with an acceptable risk of expander or implant loss.

P3-13-05
Analysis of Heart Dose-Volume Parameters and Cardiac Events among Node Positive Breast Cancer (NPBC) Patients Treated with Three-Dimensional Conformal Radiation Therapy (3D-CRT).


Background: For NPBC patients the use of regional nodal irradiation (RNI) to the supraclavicular, axillary, internal mammary lymph nodes (IMN) in addition to the chest wall and/or breast can maximize locoregional control and improve overall survival. However, comprehensive RNI for breast cancers located on the left side has been linked to late cardiac morbidity, potentially lessening the therapeutic benefit of treatment. The optimal radiation dose-volume constraints for the heart in this setting are not fully understood. We examined NPBC patients treated with RNI using 3D-CT based radiation therapy (RT) to evaluate cardiac dose and incidence of cardiac events.

Methods: Between 2000 and 2007, 150 NPBC patients were treated with RNI following lumpectomy or mastectomy using 3D-CRT. In all cases, treatment target and normal tissue volumes were delineated on treatment CT scans. The heart contour included the ventricles and the left atrium. The dose-volume histogram of the cardiac doses delivered and the incidence of cardiac events is reported.

Results: Median follow-up of surviving patients is 7 (1-10.6) years. Median patient age is 50 (27-91). 52.35% are premenopausal, 75.7% estrogen receptor positive, 66.3% progesterone receptor positive and 15.92% HER-2 positive. Mean positive lymph nodes is 5 (1-29). Extracapsular extension is present in 47.31%. Mean microscopic tumor size is 3.73 (0.1-21) cm. The IMN receive > 40 Gy in 65.5%. 94% had chemotherapy, and in 82.3% it was anthracycline-based. At the time of RT, 12.2% smoked, 9.5% had diabetes, 32.4% with hypertension, and 4.7% with a history of coronary artery disease. There was 1 (0.7%) right sided patient with cardiac events and 4 (2.7%) left sided experiencing cardiac events (p = 0.121, Fisher’s Exact test). A total of 10 cardiac diagnoses were experienced among the 5 patients: coronary artery disease with myocardial infarction (3), congestive heart failure (2), cardiomyopathy (2), and arrhythmia (3). The median time interval to onset of the events is 2.5 years (0-4.3 years).

The cardiac doses among 150 patients are as follows: mean V25 is 5.7, (0.0 - 20.0%), V25 is < 9 % in 74.4% of patients, mean V45 is 1.8% (0-13.3%), V45 is < 5.5% in 91.8%. The mean maximum point dose is 42.8 Gy, and the mean heart dose is 5.6 Gy (0.2-25.3 Gy). The mean V25 and V45 in those 5 patients with a cardiac event is 6.7% (0.9-11.9%) and 3.7% (0-6.6%), respectively; in the 145 remaining patients, 5.7% (0-20.0%) and 1.7% (0-13.3%), respectively. The mean heart dose in those with an event is 5.2 Gy (2.4-7.3 Gy) versus 5.6 Gy (0.2-25.3 Gy) in the remaining patients.

Conclusions: The cardiac event rate among these NPBC patients treated with RNI and anthracycline-based chemotherapy is low. However, those patients with cardiac events have a higher mean V45. No other dose-volume relationships are discernible. Additional analysis using 3DCRT volumes are important to validate these findings and better define the dose-volume parameters for cardiac toxicity.

P3-13-06
Cardiovascular Magnetic Resonance Imaging and Radiation-Induced Heart Disease Following Radiotherapy for Breast Cancer.


Background
Radiotherapy (RT) forms a vital component of treatment for breast cancer, contributing to the increasing number of survivors worldwide. It is known from historical cohorts that breast RT increased the risk of developing heart disease 10 years or more following treatment. It is less certain whether the lower cardiac radiation doses received during modern RT still increase cardiac risk. Cardiovascular Magnetic Resonance (CMR) imaging has the potential to provide early surrogate markers of radiation-induced heart disease that may help predict which women will be at increased risk in the future.
Methods
This is a prospective longitudinal imaging study to evaluate CMR findings in a cohort of women receiving adjuvant RT for early breast cancer at the Oxford Cancer Centre. The patients undergo CMR imaging (with gadolinium enhancement and adenosine stress), Doppler echocardiography, blood testing (including BNP and troponin), ECG and clinical examination prior to RT, within 72 hours of completing RT, and at 3 months, 6 months and 5 years following RT. The results of these investigations will be related to cardiac radiation exposure estimated using 3D-dosimetric data obtained by analysis of CT-based RT planning.

Recruitment and Provisional Results
As of May 2011, 30 women have been recruited. The women had a mean age of 57.1 years (range = 42.0 to 63.9). All women had left-sided cancer and none received adjuvant cytotoxic chemotherapy. The mean whole heart dose was 1.47 Gray (range = 0.65 to 4.20), the mean left ventricle dose was 2.03 (range = 0.88 to 6.99) and the mean left coronary artery dose was 7.55 Gray (range = 1.50 to 24.59). Preliminary analysis of the first 10 participants showed that all had a normal left ventricular ejection fraction at baseline (mean 76%, range 60-82%) and immediately following RT (mean reduction 3%, p=0.18). None had myocardial oedema as detected by T2-STIR imaging or fibrosis and scarring as demonstrated by late gadolinium enhancement (LGE).

Conclusion and Future Plans
Preliminary results suggest that the relatively low heart doses received by the women undergoing modern CT-planned left breast RT at the Oxford Cancer Centre do not result in any cardiovascular abnormality detectable by CMR imaging. Further and updated results will be available for the San Antonio Breast Cancer Symposium. Recruitment will continue until at least 20 women have completed baseline scans and follow-up scans up to 6 months. At this stage a full analysis will be performed including a wider range of CMR endpoints including:
- Overall LV systolic function (EF and volumes)
- Regional wall motion analysis
- T2-weighted imaging (oedema)
- T1 and T2 mapping (quantitative T1 and T2 relaxation times)
- Tagged myocardial strain analysis
- Stress perfusion defects (endothelial/microcapillary damage)
- Late gadolinium (LGE) imaging (fibrosis/scarring)

Depending on the results of this analysis, recruitment may be expanded to include women with either lower cardiac risk (e.g. with right-sided breast cancer) or higher cardiac risk (e.g. receiving anthracycline chemotherapy).

P3-13-07
The TARGIT-A Trial Update Confirms No Increase in Local Recurrence.
Vaidya JS, Baum M, Wenz F, Bulsara M, Tobias J, Alvarado M, Saunders C, Williams N, Joseph D, On Behalf of the TARGIT Trialists Group. University College London, London, United Kingdom; University of Heidelberg, Mannheim, United Kingdom; University of Notre Dame, Fremantle, United Kingdom; University College Hospital and Whittington Hospital, London, United Kingdom; School of Surgery, University of Western Australia, London (All), United Kingdom; University of San Francisco, London (All), United Kingdom; Sir Charles Gairdner Hospital, London (All), United Kingdom

Introduction
In June 2010, we published the results of the TARGIT-A trial (1) that compared “one-size fits” all radiotherapy (whole breast radiotherapy-E/BRT group) with risk -adjusted radiotherapy (TARGIT- group: single dose TARGeted Intraoperative radion Therapy with additional whole breast radiotherapy if adverse prognostic factors were found). These results showed that the risk of local recurrence in the TARGIT group was non-inferior to that of the EBRT group (the difference between the two arms was 0.25% at 4 years). We now report the results analyzed after further follow up of the total trial cohort without unblinding.

Method
As often repeated, unplanned analysis comparing two groups in a randomized trial can result in an increased alpha-spent as well as carry the risk of a false positive result, we have remained blinded to the further recurrences in the trial according to allocated treatment, since the original publication in the Lancet. Instead we proposed and performed a blinded analysis of the local recurrence rate for the whole cohort. We plotted the Kaplan Meier plots and compared the estimated 4-year recurrences.

Results
Amongst the 2232 patients randomized, there were 13 recurrences at the time of Lancet publication and since then, we have had 8 additional recurrences. The number of patients who have completed at least 4 years of follow up has increased from 420 to 717. We found that the 4-year Kaplan Meier estimate of local recurrence was 1.08% (95% CI 0.59 -1.96) at the time of the Lancet publication and it is 1.09% (95% CI 0.65 - 1.85) now.

Conclusion
We found that the overall 4-year recurrence rates of the TARGIT-A trial have remained stable with a longer follow up and therefore; it is statistically implausible, that one particular arm has a significantly higher local recurrence.

References
P3-13-08
Survival Analysis and Recurrence Patterns in Locally Advanced Breast Cancer Following Neoadjuvant Chemotherapy and Preoperative Concurrent Chemo Radiotherapy.
Shaw RJ, Lara F, Robles CD, Vilari D. Instituto Nacional de Cancerologia, Mexico City, Mexico

Introduction: Although not widely used, preoperative concurrent chemo radiotherapy (CCRT) following poor response to neo adjuvant chemotherapy (CT) has been recently associated with an improved prognosis in patients with locally advanced breast cancer (LABC) and high recurrence risk, showing acceptable toxicity profiles, acceptable post surgical complication rates and increases in both clinical and pathologic response rates and local control rates. The objective of this study was to assess disease free survival (DFS), overall survival (OS) and patterns of recurrence in a cohort of patients with LABC treated with pre operative CCRT following poor response to neo adjuvant CT.

Methods: This longitudinal study included a total of 287 patients with LABC treated with pre operative CCRT following poor response to neo adjuvant CT between February 2000 and December 2002. Demographic, clinical/pathologic, therapeutic and follow up data were collected from patient charts to determine DFS, OS and recurrence patterns.

Results: Stages IIB, IIIA and IIIB accounted for 22.3, 37.6 and 40% of the population, respectively. Clinical response rates compared to pathologic response rates following anthracycline based CT and CCRT were minimal or stable in 16% vs 62.4%, partial in 53 vs 76.3% and complete in 30 vs 23.3%. No significant treatment related toxicities were identified. Post surgical complications included flap ischemia in 16%, partial flap necrosis in 18%, both ischemia and partial flap necrosis in 13.2% and partial wound dehiscence in 7.7%. With an average follow up of 64.1±37.2 months, (0-126.3 months), 36.9% of the population relapsed and 55.1% remained disease free. With regard to recurrence patterns, 22.6% presented loco regional relapse, 67.7% recurred at a distant site and 9.8% showed presented both. Median OS was 64.2 months (0-126 months). Five and ten year survival probability was shown to be 75% and 60%, respectively.

Conclusions: Despite improvements in local control rates and overall outcomes with current therapeutic regimens combining chemotherapy, surgery, and radiotherapy, 30 to 40% of patients with locally advanced breast cancer develop post treatment loco regional recurrence and 5 year overall survival remains low (50%). Multimodal treatment integrating pre operative CCRT is therefore a valid alternative in the LABC setting, showing promising results in regard to OS, DFS and recurrence rates. Prospective clinical studies to evaluate its use are warranted.

P3-13-09
Impact of Estrogen Receptor Negativity on Clinical Outcomes Following Accelerated Partial Breast Irradiation.
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Purpose: To determine the impact of estrogen receptor (ER) negativity on clinical outcomes for patients treated with Accelerated Partial Breast Irradiation (APBI).

Materials/Methods(s): We evaluated 506 consecutive patients treated with interstitial brachytherapy (n=199), balloon-based brachytherapy (n=203), and 3D-CRT (n=104). ER negative (ERN) status was assigned using the traditional definition of an ER nuclear IHC stain < 10%, which corresponds to an Allred/NSABP staining score of < 10%, which corresponds to an Allred/NSABP staining score of <

2.63 patients (12.5%) were ER negative and 443 (87.5%) were ER positive (ERP). Patient demographics and clinical outcomes (IBTR, RNF, DM, DFS, CSS, OS) were analyzed for each group.

Results: The two groups had similar patient characteristics. Tumor sizes were slightly larger for the ERP group at 11.9mm vs. 10.7mm, although this was not statistically significant (p=0.14). No differences were seen in median age (63 vs. 64 years, p=0.36), rate of HER-2/neu overexpression (83% vs. 91%, p=0.11), or lymph node positivity (6% vs. 9%, p=0.55) between the ERP vs. ERP groups, respectively. There were an equal distribution of invasive ductal carcinoma (ERP n=55, 87%; ERP n=387, 87%) and DCIS (ERN n=8, 13%; ERP n=56, 13%) patients within each group. The use of chemotherapy (55% vs. 15%, p<0.001) and nuclear grade (71% vs. 12%, p<0.001) were higher in the ERP vs. ERP cohort. With a mean follow up of 6.1 years, the 5-year actuarial rates of ipsilateral breast tumor recurrence (IBTR), regional nodal failure (RNF), and distant metastasis (DM) for the entire cohort were 1.8%, 0.6%, and 3.2%. Although this was not statistically significant, ERP patients appear to have an increased rate of local failure than patients with ERP histology (4.0% vs. 1.5%, p=0.13). Rates of RNF and DM were, however, significantly higher for the ERP group (RNF: 4.9% ERP vs. 0% ERP, p<0.001; DM: 12.1% ERP vs. 2.0% ERP, p<0.001). Although there was no difference in overall survival at six years (86% vs. 90%, p=0.67), we observed a shorter disease-free survival (86.4% vs. 96.5%, p=0.01) and cause-specific survival (90% vs. 98%, p=0.01) for the ERP vs. ERP groups.

Conclusion: The ER negative phenotype of early-stage breast cancer may have a decreased rate of locoregional control. We observed a higher rate of DM with reduced disease-free and cause-specific survival in ERP negative cases, emphasizing the importance of systemic therapy and careful, long-term follow up for these patients. Prospective study of this histologic subtype with a larger cohort of patients is needed to substantiate these findings.

P3-13-10
Does Lapatinib Increase Pulmonary Toxicity When Concurrently Used with Radiation Therapy? An Experimental Study with Wistar-Albino Rats.

Purpose: Lapatinib (L) is an oral receptor tyrosine kinase inhibitor which has shown activity in the treatment of metastatic breast cancer. Adjuvant usage of L is being investigated in clinical phase III trials. There is no data regarding the side effects of combination of RT and L which has shown activity in the treatment of metastatic breast cancer. Adjuvant usage of L is being investigated in clinical phase III trials. There is no data regarding the side effects of combination of RT and L which may be a problem when L is used in the adjuvant setting. Lung is the most radiosensitive organ to observe late effects of RT. We evaluated if concurrent administration of L has any impact for the development of radiation induced pulmonary fibrosis in rats (RIPF).

Material Methods: 40 female Wistar-albino rats (WAR) were divided into 4 experimental groups (G). G1 (control) did not receive any treatment. G2 (RT) received RT to whole thoracic region. G3 (L) received L without RT. G4 (L+RT) received L with RT. A total dose of 30 Gy in 10 fractions was given to both lungs with an anterior field at 2 cm depth. L equivalent to 1500 mg/day, 60 kg adult dose, were calculated according to the mean weight of rats, orally administrated with a feeding tube twice daily including the week-ends until WAR were sacrificed. WAR were anesthetized and sacrificed 16 weeks after RT which was shown to be a sufficient period for the development of RIPF in rats. Paraffin sections (5 µm thick) of lungs were stained with hematoxylin-eosin and Masson’s trichrome. A comparative analysis was performed among 4 groups by scoring the pulmonary injury...
between 0 and 3 according to the infiltration of inflammatory cells into the alveolar spaces, alveolar wall thickening and architectural deformation across the entire lung section. Normality distribution were tested, then one-way ANOVA followed by post hoc Holm-Sidak testing were used.

Results: In G2 inflammatory cell infiltration, fibrosis with damage to lung structure and formation of small fibrous masses were observed. Alveolar septa was significantly thicker than G1 (p<0.05), which revealed totally normal pulmoner structure. G3 showed minimal alveolar septal thickening and infiltration of inflammatory cells into the alveolar spaces which was not significantly different than G1. Histopathological findings in G4 were similar to those in G2 and statistically different when compared with the G1 and G3 (p<0.05).

Conclusion: Study shows that addition of L to RT does not increase RIPF in rats.

P3-13-11
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New York Presbyterian Hospital, New York, NY

Background: The role of post-mastectomy radiation therapy (PMRT) for breast cancer patients with intermediate-risk (1-3 positive nodes) disease remains controversial. This Surveillance, Epidemiology, and End Results (SEER) study investigated the impact of PMRT in the intermediate-risk breast cancer patient population on cause-specific survival (CSS) and overall survival (OS). The study also investigated the impact of estrogen receptor (ER) status, which has been reported in the SEER database since 2004, on the impact of PMRT in this patient population on CSS and OS.

Methods: The SEER database was used to identify all breast cancer patients who were treated between 2004-2007, who had undergone mastectomy, and who had T1-2 tumors and 1-3 pathologically positive lymph nodes. The status of radiation therapy, ethnicity, tumor grade, TNM stage, year of diagnosis, number of LNs sampled, number of LNs positive, and estrogen/progesterone receptor status was recorded. Patients missing pathologic or treatment-related information were excluded. Statistical analysis for CSS and OS was performed using the Kaplan-Meier method and analyzed using the log-rank test. Multivariable analysis was performed using the Cox proportional hazards regression model. Statistical analyses were performed using PASW, version 18.

Results: 10,517 patients were identified, with a median follow-up of 21.8 months (range 0-47 months). 2339 patients (22.2%) received PMRT. Younger patients, increasing tumor size, increasing tumor grade, and ER negative tumors were more likely to receive PMRT. PMRT was associated with improved OS, 92.8% (95% CI 91.2-94.4%) versus 88.7% (95% CI 87.3-89.7%), p < 0.001. However, there was no overall CSS benefit to PMRT, p = 0.197. In the subgroup with ER positive tumors, PMRT was associated with both improved OS, 95.5% (95% CI 93.9-97.1%) versus 91.0% (95% CI 90.0-92.0%), p < 0.001, and improved CSS, 97.6% (95% CI 96.6-98.8%) versus 96.1% (95% CI 95.3-96.9%), p = 0.009. Multivariable analysis showed that PMRT was significantly associated with improved survival (HR 0.744, p = 0.011); increasing age, tumor grade, ER negative tumors, increasing tumor stage, and increasing positive nodal ratio were associated with increased risk of death.

Conclusion: Patients with intermediate risk breast cancer who received PMRT have improved OS compared to those who did not receive PMRT. For patients with ER positive tumors, PMRT also appears to be associated with improved CSS. Greater consideration for post-mastectomy radiation therapy use may be warranted for this patient population.

3-Year Cause-Specific Survival

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P3-13-12
Electrons for Intraoperative Breast Radiotherapy in Selected Patients: Long-Term Results of the Montpellier Phase II Trial.
Lemanski C, Dubois JB, Gutowski M, Gourgou S, Ailleres N, Azria D. CRLC Val d'Aurelle, Montpellier, France

Background and purpose: Postoperative whole breast external radiotherapy remains the current standard of care for patients with early operable breast cancer. In the elderly, the low crude numbers of recurrence and the frequency of comorbidities urged teams to investigate the feasibility and the results of intraoperative radiotherapy (IORT) delivered in one fraction during the surgical procedure.

Material and methods: 94 patients (> 65 years old) accepted to be included in this phase II trial according to clinical and histopathology criteria and signed the informed consent. Among them, 42 presented all the inclusion criteria, namely pT0-1 pN0, non lobular invasive unifocal carcinoma, margin ≥ 2 mm (assessed during the surgical procedure) and estrogen receptors positivity. All patients were operated upon in a dedicated IORT facility (linear accelerator) located centrally among six operated rooms. After tumor removal, the surgical bed was approximated by sutures to bring the tissue within the radiation target volume. The tumor beds were completely encompassed by 4 to 6 cm circular fields using flattened applicators at a 110 cm distance. One fraction of 21 Gy was prescribed and specified at the 90% isodose using electron with energies ranging from 6 to 10 MeV. In vivo dosimetry was performed for all patients using real-time in vivo semiconductor detectors (PTW) fixed by the surgeon within the surgical bed. A 5 year adjuvant hormonotherapy was prescribed according to the recommendations.

Primary end-point was the quality index [QI] - ratio between the prescribed dose and the in vivo measured dose-. Secondary endpoints corresponded to quality of life, local recurrences, cosmetic results, specific and overall survival.

Results: Median follow-up is now 54 months [range 12-62]. Median age was 72 years [66-80] and median tumor diameter was 10 mm [3-19 mm]. 36 tumors were grade 1 or 2 and 100% of the tumors expresses estrogen receptors. All patients received the total prescribed dose. No external postoperative radiotherapy was delivered. Intraoperative dosimetry demonstrated a good accordance between the delivered and the prescribed doses for 97% of the 37 evaluable patients. No acute grade 2 or greater toxicities were observed. Scores for quality of life were collected for all patients and showed no modification between pre and posttreatment evaluations. Late cosmetic result was good to excellent. All patients are still alive and the 4 year-disease-free-survival is currently 97%. Two patients recurred: (i) one infracentimetric superficial recurrence in the border of the surgical bed and close to the skin (at 18 months) (ii) a second primary tumor in another quadrant (at 6 months). These two patients underwent salvage mastectomy and are free of disease at the time of analysis.
Conclusion: For a very selected population, these results confirm that partial breast IORT with electrons may be considered as an interesting alternative to the standard 6-weeks radiotherapy, offering therefore a safe one-step procedure treatment.

P3-14-01
Panitumumab in Combination with FEC 100 (5-Fluorouracile, Epirubicin, Cyclophosphamide) Followed by Docetaxel (T) in Patients with Operable, Triple Negative Breast Cancer (TNBC): Final Results of a Multicentre Neoadjuvant Pilot Phase II Study.
Nabholz J-M, Weher B, Gligorov J, Mouret-Reynier M-A, Tredan O, Vanlemmens L, Petit T, Mayer F, Van Praagh-Doreau I, Dubray-Longereras P, Nyl B, Ferriere J-P, Jouannaud C, Devaute H, Tubiana-Mathieu N, Abrial C, Kwiatkowski F, Planchat E, Chalabi N, Penault-Llorca F, Chollet P, Jean Perrin Comprehensive Cancer Centre, Clermont-Ferrand, France, Metropolitan; Alexis Vautrin Comprehensive Cancer Centre, Vandoeuvre les Nancy, France, Metropolitan; Tenon University Hospital, Paris, France, Metropolitan; Leon Berard Comprehensive Cancer Centre, Lyon, France, Metropolitan; Oscar Lambret Comprehensive Cancer Centre, Lille, France, Metropolitan; Paul Strauss Comprehensive Cancer Centre, Strasbourg, France, Metropolitan; Georges François Leclerc Comprehensive Cancer Centre, Dijon, France, Metropolitan; Jean Godinot Comprehensive Cancer Institute, Reims, France, Metropolitan; Limoges University Hospital, Limoges, France, Metropolitan

Background: Panitumumab is an antibody targeting the epidermal growth factor receptor (EGFR) to which a role has been suggested in TNBC. Consequently, we evaluated the combination of a standard chemotherapy (FEC 100 followed by T) with panitumumab as neoadjuvant therapy of operable TNBC.

Methods: 60 patients with stage II-IIIa disease were prospectively included in this multicentre pilot study. Systemic therapy (ST) consisted of 4 cycles of FEC 100 (500/100/500 mg/m²) q.3 weeks followed by 4 cycles of T (100 mg/m²) q.3 weeks, in combination with panitumumab (9 mg/kg) for 8 cycles q.3 weeks. All patients underwent surgery at completion of ST. Complete pathologic response (pCR) was the primary endpoint (Sataloff/J Am Coll Surg 1995; Chevallier: Am J Clin Oncol 1993), with toxicity and biologic ancillary studies as secondary endpoints.

Results: Patients characteristics are as follows: mean age 47 [27-72]; T2: 74%, T3: 26% (mean tumor size: 40 mm [20-120]); N0: 65%, N1: 28% and N2: 7%; invasive ductal carcinoma: 96%; Scarff-Bloom-Richardson Grade III: 72%, grade II: 28%. The median number of cycles was: FEC 100: 4 [2-4], T: 4 [0-4], Panitumumab: 7 [1-8]. Pathological response showed a pCR according to Sataloff’s classification of 57.1% [95% IC: 40.7-73.3] and according to Chevallier’s classification of 51.4% [95% IC: 34.8-68.0] with an overall clinical response rate of 60% (29% CR [95% IC: 43.8-76.2].

Conservative surgery was performed in 79% of cases. Skin toxicity was the main side-effect: Cutaneous toxicity grade IV: 12%, grade III: 26%, grade II: 23%. No ocular complications have been reported. Neutropenia grade IV: 23.7%; febrile neutropenia: 4.2%. Infection: 0%. Hand-foot syndrome grade III: 4%. Unusual toxicity grade IV: 2.5%, grade II: 25.5%.

Conclusions: These results suggest that Panitumumab in combination with FEC100 followed by T appears efficacious with acceptable toxicity in the neoadjuvant therapy of operable TNBC.

P3-14-02
Sequential Versus Upfront Intensified Neoadjuvant Chemotherapy in Patients with Large Resectable or Locally Advanced Breast Cancer (INTENS), Toxicity Results from a Phase III Study of the Dutch Breast Cancer Trials' Group (BOOG).
Vriens BE, Van de Vijver KK, Boetes C, van Gastel SM, Wals J, Smilde TJ, van Warmerdam LJ, van Laarhoven HW, van Spreons DJ, Borm GF, Tjan-Heijnen VC. Maastricht University Medical Centre, Maastricht, Netherlands; Comprehensive Cancer Centre the Netherlands, Nijmegen, Netherlands; Atrium Medical Centre, Heerlen, Netherlands; Jeroen Bosch Hospital, ’s Hertogenbosch, Netherlands; Catharina-Hospital, Eindhoven, Netherlands; Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands; Canisia-Willheltina Hospital, Nijmegen, Netherlands

Background: Taxanes have an established role as (neo-)adjuvant treatment of breast cancer. In the present study, we compared 4 AC - 4 T with 6 cycles of TAC in the neo-adjuvant setting (A=adriamycine, C=cyclophosphamide, T=docetaxel). Previously, we reported that AC-T resulted in a trend for improved outcome (odds ratio pCR of the breast 1.61; 95% CI 0.79-3.33). Now we report the safety data.

Methods: Women presenting with breast cancer, cT2≥3cm, cT3, cT4 and/or cN+, with measurable disease and no prior treatment, age ≥18 and ≤70 years and Karnofsky Score ≥70% were eligible. Patients were randomized to AC (60/600 mg/m² q3wk x 4 cycles) followed by T (100 mg/m² q3wk x 4 cycles) without primary G-CSF prophylaxis, or to TAC (75/50/500 mg/m² q3wk x 6 cycles) with primary G-CSF prophylaxis. If indicated, trastuzumab and/or endocrine therapy were given as adjuvant treatment. This present analysis focuses on the toxicity profile of the two treatment arms.

Results: In total, 201 patients (n=100 AC-T, n=101 TAC) were included between February 2006 and April 2009. Baseline characteristics (AC-T/TAC) were well balanced. Patients in the AC-T arm had more frequently grade 3 / 4 toxicities as compared to the TAC arm, respectively in 57% and 28% (p=0.001). Grade 3 / 4 neutropenia without fever was more frequently reported with AC-T (35% vs. 4%; p=0.001). Grade 3 / 4 febrile neutropenia was also more frequent with AC-T (17% versus 5%; p=0.0062) and significantly increased during docetaxel treatment after AC. Notably, diarrhea was also more frequently seen in the AC-T arm (4% versus 0%, p=0.0423). Other grade 3 / 4 toxicities more frequently reported in the AC-T arm were neuropathy – sensory (5% vs. 0%; p=0.229) and pain other than muscle or bone pain (4% vs. 0%; p=0.0423). There were no grade 3 / 4 toxicities more frequently observed in the TAC arm.

Conclusion: In the comparison of two taxane-anthracycline-cyclophosphamide regimens in the neo-adjuvant setting, it is observed that the sequential approach with a lower cumulative dose tends to have a slightly better efficacy outcome, but at the cost of increased grade 3 / 4 toxicity. However, considering the use of primary G-CSF prophylaxis in the TAC arm, and the fact that the incidence of febrile neutropenia was higher during taxane containing chemotherapy in the AC-T arm, the difference might (partly) disappear if primary G-CSF prophylaxis would be used in the sequential arm. Primary G-CSF prophylaxis may be considered during docetaxel if used sequentially after anthracycline-containing chemotherapy.

Support: Unrestricted grants from sanofi-aventis NL BV and Amgen BV.
P3-14-03
ABCB1 Single Nucleotide Polymorphisms as a Possible Prognostic Factor in Breast Cancer Patients Receiving Docetaxel and Doxorubicin Neoadjuvant Chemotherapy on Systemic Treatment.

Background
Expression of the adenosine triphosphate-binding cassette B1 (ABCB1) transporter and P-glycoprotein are associated with resistance to anticancer drugs. The purpose of this thesis was to investigate the role of single nucleotide polymorphism (SNP) in the ABCB1 and CYP3A genes in breast cancer patients who were treated with neoadjuvant docetaxel and doxorubicin chemotherapy.

Material and Methods
Patients with histologically confirmed breast cancer, Stage II or III, referred for neoadjuvant chemotherapy were enrolled. Patients were treated with 3 cycles of neoadjuvant and adjuvant chemotherapy with docetaxel and doxorubicin. The polymorphisms of ABCB1 (C3435T, G2677T/A, and C1236T) were genotyped by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) assays. The genotyping of CYP3A was done by the GoldenGate assay (Illumina Inc). The correlation of genetic polymorphisms of ABCB1, CYP3A, and clinical outcomes was analyzed.

Results
A total of 216 patients were enrolled, and the median age was 44 years (range 25 - 69 years). The overall radiologic response rate (RR) was 76.8% whereas 8.3% of the patients achieved a pathologically complete response. After a follow-up duration of 48.8 months, the median relapse-free survival (RFS) and overall survival (OS) were not reached. ABCB1 3435TT genotype had a longer OS than CT/TT genotype (p=0.045) and a trend toward a lower relapse rate (p=0.284) although it is statistically insignificant. With univariate analysis of the OS, good performance status (PS), invasive ductal carcinoma, initial operable stages, estrogen receptor (ER)-positive, non-triple negative, and the TT genotype of ABCB1 C3435T were associated with a lower risk of death. Multivariate analyses for the OS revealed that PS (HR 4.670, 95% CI=1.066-10.468; p=0.041), initial clinical stage (HR 3.198, CI=1.480-6.907; p=0.003), and triple negative phenotype (HR 3.091, 95% CI=1.245-6.570; p=0.004) were significantly associated with the OS. ABCB1 3435TT genotype was also associated with a lower risk of death with marginal significance not shown to be an independent prognostic factor (HR 0.295 95% CI=0.121-1.122; p=0.071). ABCB1 3435TT genotype had a higher AUC than CC/CT genotype for docetaxel with marginal significance (p=0.054). These higher AUCs in the C3435TT genotype were associated with increased toxicities of neutropenia (p=0.037) and diarrhea (p=0.017). AA (*1/*1)/AG (*1/*3) genotypes of CYP3A5 had a higher AUC than GG (*3/*3) for docetaxel with statistical significance (p=0.024). However, these higher AUCs of CYP3A5*1 allele carrier did not affect the survival and toxicities.

Discussion
This study showed that the genetic polymorphism of ABCB1 C3435T might be associated with a longer OS and have predictive roles after the treatment with the neoadjuvant docetaxel/doxorubicin for stage II and III breast cancer. Our results also suggest that the prediction of docetaxel toxicity might be possible for ABCB1 C3435T polymorphism. Larger prospective studies as well as functional studies in human subjects are warranted.

P3-14-04
Assessment of Genomic Prognostic Signatures as Predictors of Response to Neoadjuvant Chemotherapy in Patients with Early Stage Breast Cancer.
Culakova E, Poniewierski MS, Huang M, Kuderer NM, Ginsburg GS, Barry W, Marcom PK, Ready N, Abernethy A, Lyman GH. Duke University, Durham, NC

Background: Based on results from randomized clinical trials, adjuvant and neoadjuvant chemotherapy (NCT) strategies in early stage breast cancer patients (ESBC) achieve comparable long term results. Recently, a number of genomic signatures have been reported, distinguishing patients with low versus high risk of recurrence. While developed primarily as prognostic assays, these classifiers have also been proposed to be predictive of benefit from systemic chemotherapy. Neoadjuvant studies provide an opportunity to evaluate their predictive value for response to NCT.

Methods: A systematic review of gene expression profile studies in ESBC patients receiving chemotherapy was conducted. Medline search of original research articles of human studies published between January 2000 and February 2011 was based on key words and MeSH heading terms. Publications presenting outcomes for chemotherapy treated patients in groups stratified by multi-gene array signatures and utilizing a new independent cohort of patients compared to the original development cohort were selected. Information from eligible studies was extracted by dual abstraction. Reported results were synthesized into combined diagnostic odds ratio (DOR) using method of Mantel-Haenszel. This analysis is restricted to neoadjuvant studies investigating the association of genomic signature prognostic categories with objective tumor response to chemotherapy.

Results: A total of 42 articles were eligible for data abstraction. Out of these, 6 publications evaluated response to NCT in good (low risk of recurrence) versus poor prognosis groups based on genomic prediction. Since two of the studies analyzed the same signature on a cohort with large overlap, only 5 studies were included in the final analysis, accounting for n=918 patients. Response consisted of pathologic complete response (pCR) in 3 studies, pCR or minimal residual disease (1 study), and clinical complete response (1 study).

Prognostic genomic assays included Oncotype DX (1), MammaPrint (1), Genomic Grade Index (2) and PAM50 Risk of Relapse Score (1). Eight different chemotherapy regimens were utilized. The most common drugs were cyclophosphamide, anthracyclines, taxanes, and 5-fluorouracil. Across all genomic signatures, good prognosis patients, as defined by gene expression data, demonstrated consistently low rates of response to chemotherapy (median 3%, range 0-12%) compared to patients with less favorable prognosis (median 32%, range 19-43%). Odds ratio for response in poor versus good prognosis patients ranged from 3.9 to 21.7 with combined DOR= 6.6 (95% CI 3.9-11.3, P<0.0001). No heterogeneity was determined across studies (P=0.4). The C-statistic estimating assay discriminatory ability was reported in 3 studies ranged from 0.72 to 0.78.

Conclusions: Across all genomic prognostic signatures reported, only a very small proportion of patients with signature predicted good prognosis achieved complete response to NCT. This suggests low sensitivity to chemotherapy among good prognosis patients, as determined by the prognostic genomic signatures. This further confirms the association between poor prognosis tumors and higher responsiveness to chemotherapy.

Funding: NCI: UC2CA14041-01
P3-14-05
Evaluation of Residual Cancer Burden Index (RCBI) as a Predictor of Disease Free Survival in a Non-Selected Cohort of Breast Cancer Patients Treated in the Neoadjuvant Setting.

INTRODUCTION: Pathologic complete response (pCR) is associated with long-term survival and is considered as the primary endpoint in neoadjuvant trials. Definition of pCR includes patients without residual disease of the breast, however the presence of nodal metastasis, minimal residual cellularity and residual in situ carcinoma were not consistently defined as pCR. These observations, lead Symmans et al to construct a new prognostic index, the RCBI, in which these important issues were incorporated. RCBI is classified into 4 different response subgroups, from RCB-0 or pCR to RCB-III or absence of response. The aim of our study was to assess the prognostic value for recurrence free survival of the RCBI in a cohort of unselected breast cancer patients treated in our institution. PATIENTS AND METHODS: We performed a retrospective evaluation of samples of breast cancer patients treated with neoadjuvant treatment. RCBI was assessed by two highly trained pathologists. Patients included had a histological diagnosis of breast carcinoma before neoadjuvant treatment and were considered candidate for neoadjuvant therapy. Clinical variables including date and type of recurrence were obtained from clinical records. Prognostic accuracy of RCBI index was evaluated by comparison of the Kaplan-Meier survival curves of the 4 different groups of response by RCBI. Differences were assessed with log-rank. RESULTS: Samples from 70 patients treated in the neoadjuvant setting from January 2003 to December 2006 were included in the analysis. Median age was 54.6 years (range 31-80), histologic subtype was ductal carcinoma (87.1%), lobular carcinoma (8.5%), and other (4.2%). In biopsy, rate of estrogen receptor positive was 72.7%, progesterone receptor positive 62.1%, and rate of HER2 overexpression was 26.2%. Neoadjuvant therapy administered was anthracycline-taxanes chemotherapy (CT) in both sequential or combination schedules in 71.4%, anthracycline-based CT in 15.7%, and other in 12.9%. Most patients received from 6-10 cycles of neoadjuvant CT. 3 patients treated with only 4 cycles received adjuvant complementary CT. All patients with hormone-sensitive tumors received adjuvant endocrine therapy and 20 patients received adjuvant trastuzumab. Number of responses by RCB-group was 5 patients (7.1%) with RCB-0, 12 patients (17.1%) with RCB-I, 35 (50%) with RCB-II, and 18 (25.7%) with RCB-III. With a median follow-up of 41.2 months (range 12.7-120.9 months) 22 patients (31.4%) have relapsed. Ratio of events/patients in each RCB group was 0/5 in RCB-0, 2/12 in RCB-I, 8/35 in RCB-II and 15/18 in RCB-III. Differences in recurrence-free survival in each group was statistically significant with p<0.0002. CONCLUSIONS: Our results suggest that RCBI might be an appropriate prognostic tool to predict disease-free survival in a non-selected population of breast cancer patients. Further validation of our results with a bigger sample size is needed.

P3-14-06
Combined Use of 18F-FDG PET/CT and MRI To Monitor Breast Cancer Response during Neoadjuvant Chemotherapy.

INTRODUCTION: Pathologic complete response (pCR) is commonly used for the evaluation of the therapeutic effect of neoadjuvant chemotherapy (NAC). A recent study demonstrated the relevance of breast cancer subtype with regard to the accuracy of MRI to predict response during NAC. The role of 18F-fluorodeoxyglucose (FDG) positron emission tomography (FDG PET/CT) is under investigation, and varying results have been reported. MRI and 18F-FDG PET/CT, visualize different functional characteristics of the tumor (perfusion and glucose metabolism), but it is unclear if they complement one another to monitor response to NAC, and whether breast cancer subtype plays an independent role.

Method and material
The study group consisted of 65 women with stage II or III breast cancer and measurable FDG tumor uptake, who were treated with NAC and participated in an ongoing MRI/FDG PET/CT study. MRI and FDG PET/CT scans were acquired prior to and during treatment, using prone patient positioning. Written informed consent was obtained. Prior to the start of NAC a biopsy was taken and tumors were divided into three subtypes, using immunohistochemistry: human epidermal growth factor receptor 2 (HER2) amplified (HER2+)(N=18), estrogen receptor positive/HER2 non-amplified (ER+/HER2-)(N=29), and triple negative (TN) tumors (N=18). MRI interpretation included lesion morphology at baseline, changes in morphology, tumor size, and contrast uptake kinetics (initial and late enhancement). At FDG PET/CT the maximum standardized uptake value (SUVmax) at baseline and during treatment was obtained. Tumor response pathologically was stratified to “incomplete response” (substantial presence of vital invasive tumor cells) and “favourable response” (complete absence of invasive residual tumor or only a small number of scattered tumor cells). Appropriate statistical analyses including T-tests, multivariate logistic regression and receiver operating characteristic (ROC) curves were employed to indentify factors associated with incomplete response at pathology. The following imaging and clinical features were candidates for forward feature selection in the multivariate analysis: lesion morphology, largest tumor diameter, pattern of reduction, absolute and relative change in largest tumor diameter at initial and at late enhancement during NAC, SUVmax at baseline; absolute and relative change in SUVmax during NAC; breast cancer subtype; chemotherapy regimen, and age.

Results
At pathology after NAC, 32 tumors (49 %) showed favourable response and 33 (51 %) had residual disease. At multivariate analysis, breast cancer subtype, relative change in the diameter of late enhancement on MRI and relative change in SUVmax on FDG PET/CT were independent markers of tumor response at final pathology. The area under the ROC curve increased from 0.86 for MRI alone to 0.94 (p<0.01) in combination with FDG PET/CT and breast cancer subtype, yielding 84% sensitivity at 95 % specificity.

Conclusion
MRI and FDG PET/CT show complementary potential to predict response during NAC, in addition to breast cancer subtype.

Acknowledgement: This study was supported by the Center for Translational Molecular Medicine, project Breast CARE (grant 03O-104).
P3-14-07
Relative Risk of Recurrence (RR) over Time in ER Positive and Negative T4 Breast Cancer Patients Achieving Less Than pCR (<pCR) after Primary Chemotherapy: A Reversal Trend of Recurrence beyond 60 Months after Diagnosis.
Ionta MT, Atzori F, Pascuccio V, Notari F, Valle E, Guerzoni D, Chiappe A, Marongiu M, Minerba L, Massidda B. University Hospital, Cagliari, Italy; Hospital of Oncology Businco, Cagliari, Italy

Background: It is widely assumed that patients (pts) who achieve a pCR have a good prognosis and better outcomes compared with pts with <pCR, regardless of hormone receptor status. Emerging data suggest that achieving < pCR identifies a heterogeneous group with different risks of recurrence and death, being ER negative the subgroup with the worst prognosis.

Aim of our study was to evaluate the relative risk of recurrence (RR) over time in ER-positive (ER+) and ER-negative (ER-) <pCR patients.

Material and Methods: We analyzed 139 consecutive <pCR T4 pts, of whom 85 were ER+ (cut-off 10%) and 54 were ER-. Median age was 53 y (range 29-73). Median follow up was 137 months (range 8-233). All pts received primary anthra-based chemotherapy with or without taxanes followed by endocrine therapy for 5 years, if hormone receptor positive. We examined the RR of recurrence at 0-24, 25-60, 61-120 and beyond 120 months (m) interval after diagnosis.

Results: For all 139 pts, the total number of pts with recurrence was 95 (68%), 54 (63%) in ER+ and 41 (76%) in ER- tumors. For the entire group and both in ER+ and in ER-, the RR of recurrence was greatest for the interval between 0-24 months, then decreased rapidly in ER- and slowly in ER+ through the 25-120 m interval. For the 0-24 m and 25-60 m intervals the RR of recurrence was higher for ER- pts and after 60 m it crossed and beyond 60 m the RR of recurrence was higher in ER+ than in ER- pts. Notably, beyond 120 months we observed a little second peak of recurrence, higher in ER+ (7%) than in ER- (3%) pts. Nine of these 10 relapses occurred between 120 to 180 m interval, 7 in ER+ (13%) and 2 in ER- (5%) subgroup. In the first 24 m ER- pts were 86% more likely to relapse compared with ER+ vice versa between 61-120 and beyond 120 m intervals, ER- pts were 19% and 18% less likely to relapse, respectively, compared with ER+ pts.

Conclusions: The present study confirmed the previous reports which showed unfavorable prognosis of the <pCR patients with negative ER status, due to their significantly higher relative risk of recurrence between 0 to 60 months interval. The ER+ recurrences occurred more frequently in late follow-up. A reversal of recurrence between ER positive and negative patients beyond 60 months after diagnosis was detected. The fact might indicate the importance of long term adjuvant hormone therapy for T4 ER positive breast cancer patients with <pCR after primary chemotherapy.

Global Recurrences/ pts at risk
<table>
<thead>
<tr>
<th>N. (%)</th>
<th>0-24 m (RR)</th>
<th>25-60 m (RR)</th>
<th>61-120 m (RR)</th>
<th>&gt;120 m (RR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>139 (68)</td>
<td>22/139 (1.3)</td>
<td>111/144 (0.50)</td>
<td>10/88 (1.1)</td>
<td></td>
</tr>
<tr>
<td>52 (13%)</td>
<td>5/52 (0.49)</td>
<td>8/53 (0.75)</td>
<td>4/48 (0.40)</td>
<td>3/45 (0.57)</td>
</tr>
</tbody>
</table>

P3-14-08
A Phase II Study of Gemcitabine and Carboplatin (GC) Plus Iniparib (BSI-201) as Neoadjuvant Therapy for Triple-Negative and BRCA1/2 Mutation-Associated Breast Cancer.

Background: The combination of gemcitabine, platinum and inhibition of DNA repair results in synergistic chemosensitivity in triple-negative and BRCA1-deficient breast cancer cell lines. This study was designed to assess efficacy, safety and predictors of response to iniparib, a small molecule whose mechanism of action is under investigation, in combination with GC in early-stage triple-negative and BRCA1/2 mutation-associated BC.

Methods: This single-arm, phase II study (NCT00813956) enrolled patients with clinical stage I–IIIA (T ≥1cm by MRI) estrogen receptor-negative (≤5%), progesterone receptor-negative (≤5%), and HER2-negative or BRCA1/2 mutation-associated BC. Neoadjuvant gemcitabine (1000 mg/m2; IV; D1, 8), carboplatin (AUC 2; IV; D1, 8), and iniparib (5.6 mg/kg; IV; D1, 4, 8, 11) were given every 21 days for 4 cycles, until the protocol was amended to increase the treatment duration to 6 cycles with enrollment of 36 patients. The protocol was further amended to increase the patient number with expansion to additional centers. Prior to expansion, reporting of interim safety and efficacy results was specified. The primary endpoint is pathologic complete response (pCR), defined as the absence of invasive carcinoma in the breast and axilla. Pathologic response was assessed using the residual cancer burden (RCB) index. Secondary endpoints included safety, radiographic response by MRI, breast conservation eligibility and correlation of baseline gene expression with response.

Results: The pre-expansion cohort consisted of 13 patients assigned to 4 cycles of treatment and 36 patients assigned to 6 cycles of treatment. Among these 49 patients (50 tumors), median tumor size is 35 mm, and median age is 48 years. The overall pCR rate is 34% (95% CI = 22-48); the pCR rate at 6 cycles is 41% (95% CI = 26-57). RCB 0/I was observed in 5/13 pts (38%; 95% CI = 18–64) treated with 4 cycles; 21/37 (57%; 95% CI = 41-71) treated with 6 cycles; and 9/9 pts (100%) with known BRCA1/2 mutations. Two pts experienced disease progression, two locoregional relapse, and three distant relapse. Among 45 tumors with Affymetrix-based RNA microarray gene expression data, 39/45 (87%) are classified as basa-like, 3/45 (7%) luminal B, and 3/45 (7%) normal breast-like. Most common G3/4 adverse events include neutropenia (31%), anemia (10%), and elevated ALT/AST (10%). Alopeica and chemotherapy-induced amenorrhea were uncommon. Analyses of secondary endpoints are ongoing and will be presented.

Conclusions: Preoperative GC plus iniparib is active in the treatment of early-stage triple-negative and BRCA1/2 mutation-associated breast cancer.
P3-14-09
A Phase II Preoperative Study of Dasatinib, a Multi-Targeted Tyrosine Kinase Inhibitor, in Locally Advanced “Triple-Negative” Breast Cancer Patients.
Rimawi MF, Rodriguez AA, Yang WT, Gonzalez-Angulo AM, Nangia JR, Wang T, Speers C, Mills G, Hilsenbeck SG, Brown PH, Chang JC. Baylor College of Medicine, Houston, TX; The Methodist Hospital, Houston, TX; M.D. Anderson Cancer Center, Houston, TX
Background: We previously reported that kinases (Src, Yes-1, cKIT, Abl, and EPH4) were druggable in triple negative breast cancer (TNBC). In this clinical trial, we sought to translate these findings by treating TNBC patients with dasatinib, a multi-targeted kinase inhibitor against these targets.
Methods: Women with stage II-III TNBC were eligible. Patients received dasatinib at 100 mg daily for 3 to 4 weeks before standard-of-care definitive surgery and chemotherapy. Biopsies were performed at baseline, week 1, and at the time of surgery. A cohort of patients had positron emission mammography (PEM; baseline and at 2-3 weeks of dasatinib therapy). This study was designed to detect an increase in clinical response rate from 10% to 25%, using a Simon optimal two stage design, with one-sided alpha=5% and power=80%. At least 3 responses out of 22 patients were needed to proceed to the second stage.
Results: 22 patients were enrolled (Table 1). Median tumor size was 7.0 cm (range 2.4-25 cm). Adverse events were modest, mainly grade 1-2 (headache: 45%, abnormal LFTs: 55%, GI: 23%, fatigue: 18%). One patient had a myocardial infarction 24 hours after starting dasatinib. Out of 22 patients, 2 (9%) had a clinical partial response after 3-4 weeks of therapy, 15 had stable disease (68%), while 5 had progressive disease (23%). Of the 8 patients who received paired PEM imaging, metabolic responses were observed in 2 patients (25%). Conclusion: A short course of dasatinib led to clinical responses in 2 out of 22 patients with TNBC, and the study did not proceed to second stage. Since TNBC is a heterogeneous disease, biomarker studies including sequencing of candidate genes like B-RAF for inactivating mutations might enable selection of those TNBC patients who could benefit from dasatinib.

Table 1: Patient Characteristics
<table>
<thead>
<tr>
<th>Age</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>47 years (28-79)</td>
<td>15</td>
<td>68</td>
</tr>
<tr>
<td>68 years (45-79)</td>
<td>7</td>
<td>32</td>
</tr>
<tr>
<td>Hispanic</td>
<td>13</td>
<td>59</td>
</tr>
<tr>
<td>Not Hispanic</td>
<td>9</td>
<td>41</td>
</tr>
<tr>
<td>Tumor Size</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 5cm</td>
<td>7</td>
<td>32</td>
</tr>
<tr>
<td>&gt; 5 cm</td>
<td>15</td>
<td>68</td>
</tr>
</tbody>
</table>

P3-14-10
Early Predictive Value of Non-Responder to Docetaxel in Neoadjuvant Chemotherapy in Breast Cancer Using 18F-FDG-PET.
Hirakata T, Fujisawa T, Yanagita Y, Horikoshi H, Oya N, Akiyoshi T, Kinoshita T, Kikuno H. Gunma Prefectural Cancer Center, Ota, Gunma, Japan; Tsurugaya Hospital, Iseaki, Gunma, Japan; Graduate School of Medicine, Gunma University, Maebashi, Gunma, Japan
Background: Clinical response is determined after several cycles of chemotherapy by changes in tumor size in imaging procedures including ultrasound, MRI or CT. The aim of this prospective study was to early detect non-respondent to docetaxel (DTX) in neoadjuvant chemotherapy using 18F-FDG-PET in patients with breast cancer.
Method: 41 patients were eligible for this study. 37 were assessable (4 were not eligible or not evaluable) with Invasive carcinoma (T1:10%, T2: 83%). All 37 patients were treated with 4 cycles of docetaxel (75mg per square meter) followed by 4 cycles of FEC(cyclophosphamide/epirubicin/fluorouracil: 500/75/500 mg per square meter) before surgery. 18F-FDG-PET response rate was evaluated between before and after the first cycle of DTX. 18F-FDG-PET images were analyzed by standardized uptake value, SUVmax. Clinical response is determined by reduction rate in tumor size with MRI (RECIST criteria) between before the first cycle and after the fourth cycle of DTX. Pathological response evaluated by core needle biopsy after the fourth cycle of DTX.
Result: 18F-FDG-PET response rate after the first cycle of DTX showed correlation with tumor size reduction rate with MRI image after the fourth cycle of DTX (r=0.746)(P<.001). 0 out of 8 patients (0%) revealed cPR with MRI changes in SUV decrease less than 18% (P <.001). 8 out of 13 patients (62%) showed cPR (cCR = 0) with SUV in SUV decrease over 19 to 44% (P <.001). 9 out of 16 patients (56%) were cPR, and 7 out of them (44%) showed cCR with MRI in SUV decrease over 45% (P <.001).
Conclusion: Changes of 18F-FDG SUVs in tumors were statistically significantly different between responding and nonresponding (P <.001). SUV decrease less than 18% after the first cycle indicated potential failure to DTX in neoadjuvant chemotherapy.

P3-14-11
Comparison of Two Nomograms To Predict Pathologic Complete Response to Neoadjuvant Chemotherapy – Evidence That HER2 Positive Tumors Need Specific Predictors.
Aim: The purpose of this study was to compare two different nomograms to predict pathologic complete response (pCR) to preoperative chemotherapy in an independent cohort of 200 patients with breast cancer. The first model was the MDACC nomogram published in 2005 and the other one was a nomogram based on the number of preoperative courses, Ki-67 and steroid hormone receptors expression published by Colleoni et al. in 2010
Patients and methods: Data from 200 patients with breast carcinoma treated with preoperative chemotherapy and operated at Tenon Hospital from 2001 to 2009 were collected. We calculated pCR rate predictions with the two nomograms and compare those predictions with outcome. Patients received between 4 and 8 course of anthracycline/taxanes based chemotherapy. More than 90% of patients with HER2 positive tumors received concomitant trastuzumab with taxanes. Model performances were quantified with respect to discrimination (evaluated by the areas under the receiver operating characteristics curves (AUC)) and calibration.
Results: In the entire population, the AUC for the MDACC nomogram and the Colleoni nomogram were respectively 0.74 and 0.75. Both of them underestimated the pCR rate (p=0.02 and 0.0005). When excluding patients treated with trastuzumab, the AUC were 0.78 for both of them with no significant difference between the predicted and the observed pCR (p=0.14 and 0.15). When analyzing the specific population treated with trastuzumab as preoperative treatment, the AUC for the MDACC nomogram and the Colleoni nomogram were respectively 0.52 and 0.53.
Conclusion: The MDACC and the Colleoni nomograms are accurate to predict the probability of pCR after preoperative chemotherapy in HER2 negative population but did not correctly predict pCR in
HER2 positive patients who received trastuzumab. This suggests that response to preoperative chemotherapy including trastuzumab is biologically driven and that a specific nomogram or predictor for HER2 positive patients has to be developed.

**P3-14-12**

Local Control of Primary Breast Cancer Treated with Radical Radiotherapy Alone after Neoadjuvant Chemotherapy.

Makris A, Li SP, Ravichandran D, Ostler PJ, Pittam M, Mount Vernon Cancer Centre, Northwood, Middlesex, United Kingdom; Laton and Dunstable, Laton, United Kingdom

**Introduction:** The aim of this retrospective study was to evaluate the local recurrence rates in a cohort of patients who achieved a complete clinical response (cCR) to neoadjuvant chemotherapy and did not have surgery.

**Materials and Methods:** 148 women who achieved a cCR to neoadjuvant chemotherapy were identified from a prospectively maintained database (1995-2011) of 667 patients. 122 patients went on to have surgery (either wide local excision or mastectomy) followed by radiotherapy. In 26 patients (median age 49, range 35-72 years; T2-T4, N0-3, M0), no surgery was performed but all received radical radiotherapy. Surgery was avoided due to either physician or patient choice. Recurrence was defined as first relapse of disease, either local (ipsilateral breast and/or axilla) or distant.

**Results:** All 26 patients who avoided surgery had neoadjuvant chemotherapy with 20 patients (77%) receiving anthracycline-based (FEC, FAC, ECF), 5 (19%) MMM and 1 (4%) CMF chemotherapy. The median number of cycles was 6 (range 4-8). Chemotherapy was followed by radical external beam radiotherapy to the breast +/- supraclavicular fossa and axilla (median dose delivered, 60Gy in 2Gy fractions). All were identified as operable at diagnosis including 3 patients who had supraclavicular lymphadenopathy. All 26 patients achieved a final cCR in the breast to chemotherapy. 21 patients had imaging with mammography and/or ultrasonography to assess radiological response at the end of neoadjuvant chemotherapy, 20 of which had a complete response and 2, a partial response. After a median follow-up of 144 months, 10/26 (38%) patients experienced local disease recurrence (2 also had distant recurrence) and 4/26 (15%) patients with distant metastases only. Patients with local recurrence only, went on to have a mastectomy whilst those with distant disease received systemic therapy. There were 10 deaths, 9 of which were breast cancer related (33%).

**Conclusions:** Local recurrence rates were high in patients achieving a cCR following neoadjuvant chemotherapy and who avoided surgery. Our practice has subsequently changed to include clip insertion and surgical excision on completion of chemotherapy. With increasing pathologic complete response rates to more active chemotherapy schedules (including taxanes +/- herceptin), it is being proposed that surgery could be avoided in selected patients. Our study shows that caution should be exercised.

**P3-14-13**


Llombart-Cussac A, Pernas-Simon S, Rezai M, Hauchchild M, Venturini M, Machiels J-P, Paepe S, Luporsi E, Kasiborski F, Kayitalire L, Hospital Armai de Vilanova, Lleida, Spain; L’Hospital de Llobregat, Barcelona, Spain; Luisenkrankenhaus Düsseldorf, Düsseldorf, Germany; Ospedale Sacro Cuore, Negrar (Verona), Italy; Cliniques Universitaires St-Luc, Université Catholique de Louvain, Brussels, Belgium; Technischen Universitat München, München, Germany; Centre Alexis Vautrin, Vandoeuvre-Les-Nancy, France; Cephalon, Maisons-Alfort, France; Frauenklinik und Brustzentrum Rheinfelden, Rheinfelden, Germany

**Background:** The upfront addition of trastuzumab (Herceptin, H) to anthracycline/taxane-based primary chemotherapy significantly increases pathological complete response (pCR) in HER2-positive breast cancer (BC) patients (pts). However, concerns about the cardiac toxicity of concurrent administration of H and standard anthracyclines limited its use (Seidman et al 2002, J Clin Oncol 20:1215-21). MYOCET® (M) has a better cardiac tolerance profile than standard doxorubicin (Adriamycin, A) and might allow to give anthracyclines safely in combination with H. We explored the added benefit of early and longitudinal exposure to the combination of M with H in early-stage HER2-positive BC in a phase II multicentric randomized trial.

**Methods:** Women with stage II or IIIA HER2-positive BC were randomized to receive 4 cycles of either M + cyclophosphamide (C) + H (MCH) or AC every 3 weeks (q3w); then followed by 4 cycles of docetaxel + H (q3w). The primary endpoint was the pCR rate. Secondary endpoints included the overall and cardiac safety (using left ventricular ejection fraction [LVEF] and NYHA classification).

**Results:** Since September 2008, 126 pts with a median age of 51 and stage II (85 pts) or III (39 pts) BC were randomized: 1 pt did not initiate treatment, 12 discontinued treatment prematurely, 21 were still receiving treatment, 91 had completed treatment and 90 underwent surgery, as of January 15th 2011. Pathological responses are under central review.

In the MCH arm, 1 pt reported a NYHA class II cardiac event and 1 pt had asymptomatic LVEF decrease down to 42%. In the AC arm, 2 pts experienced NYHA class II events, 1 pt had LVEF value 17% below baseline, and 1 pt had LVEF value 6% below the lower normal limit. Mean (±SD) LVEF decrease from baseline to endpoint was 0.8% (±6.3) in the MCH arm and 3.8% (±5.8) in the AC arm. Patients tended to report more grade 3 gastrointestinal (GI) adverse event in the MCH arm (8.1% of pts) than in the AC arm (4.8% of pts), mainly diarrhea (6.5% of pts in the MCH arm vs 1.6% in the AC arm). Other grade 3 GI toxicities were infrequent. Hematological toxicity was similar in both arms: 56.4% and 61.9% of pts developed grade 3/4 neutropenia, and 16.1% and 14.3% of pts reported febrile neutropenia, in the MCH and AC arms respectively. No severe case of palmar-plantar erythrodyssaesia syndrome was reported.

**Conclusion:** The concurrent administration of the non-pegylated liposomal doxorubicin MYOCET® and H has an acceptable early safety profile that should be confirmed with longer follow-up. Pathological response data are pending and will be presented at the meeting.
P3-14-14

Increased Prevalence of Low Vitamin D Level in Breast Cancer Patients during Neoadjuvant Chemotherapy.

Jacot W, Poudoures S, Thezenas S, Chapelle A, Romieu G, Lamy P-J. CRLC Val d’Aurelle, Montpellier, France

Purpose: Patients with early-stage breast cancer treated with neoadjuvant chemotherapy (NCT) are at risk of bone metabolism changes, resulting in loss of bone mass. Cancer treatment-induced bone loss (CTIBL) increases the risk for skeletal morbidity. An understanding of CTIBL is critical for determining the risk, identifying which patients may benefit from preventive therapy, and for screening patients for early intervention with therapies such as bisphosphonates or anti-RANKL therapies. In addition, considering the bone metabolism profile in early breast cancer patients, only a minority of women had vitamin D levels in the range considered sufficient for optimal health. This vitamin D deficiency may be associated with poor outcomes in breast cancer. In order to study the variation of the bone metabolism markers during NCT, we evaluated 78 early breast cancer patients homogeneously treated in our centre.

Patients and methods: We studied the serum level of a panel of bone metabolism biomarkers (calcium, vitamin D, TRAIL, RANK-Ligand, Osteoprotegerin (OPG), serum bone trap, serum crosslap and DKK1), in a population of 78 early breast cancer patients treated by the association of 3 cycles of FEC100 then 4 taxane cycles between March 2007 and August 2008. Serum samples were withdrawn before the first CT cycle and before surgery. Time course and correlations between these biomarkers levels were evaluated. All computations were carried out using Stata. Two-sided P-values below 0.05 were considered to be statistically significant.

Results: The clinicopathological characteristics of the population were classical of a neoadjuvant setting. 23% of the patients achieved a pCR. At baseline, 79.5% of the patients presented with vitamin D insufficiency (<30 ng/ml). This proportion increased to nearly all the patients before surgery (97.4% p < 0.0001). The same significant decrease in serum levels was found for calcium and RANK-ligand, while a significant increase in serum OPG level was noted, resulting in an increase of the OPG/RANK-Ligand ratio. Serum calcium and vitamin D levels were significantly correlated (Spearman’s coefficient = 0.23), while there was a trend (Spearman’s coefficient = 0.1) for correlation between serum calcium and RANK-Ligand levels.

Conclusion: To our knowledge, this is the first clinical study comprehensively evaluating the changes in bone metabolism during early stage breast cancer NCT. In our study, nearly all the patients suffered from vitamin D insufficiency by the end of NCT. This insufficiency was associated with changes in the calcium / Rank-Ligand / OPG axis, evocative of a functional regulatory mechanism. These results require further analyses in order to define the role of pharmacologic modulation of this regulatory mechanism in the bone wellbeing of early breast cancer patients. A study evaluating the best medical intervention to correct this highly prevalent vitamin D insufficiency in this population is ongoing.

P3-14-15

Non-Randomized, Open Label Phase II Trial Evaluating the Safety and Efficacy of Taxotere (T) Followed by Myocet (M) + Cyclophosphamide (C) as First-Line Treatment for HER2-Negative Breast Cancer (BC).

Garcia-Mata J, Calvo L, Lopez R, Ramos M, Castellanos J, Heras L. Complexo Hospitalario de Ourense, Ourense; Spain; Complexo Hospitalario Universitario A Coruña, A Coruña, Spain; Hospital Clínico de Santiago, Santiago de Compostela, A Coruña, Spain; Centro Oncológico de Galicia, A Coruña, Spain; Complexo Hospitalario Universitario Xeral Cies, Vigo, Pontevedra, Spain; Hospital Sociosanitari de l’Hospitalet, Barcelona, Spain

Background: Breast cancer is the most common malignancy in females in Europe and is the most common cause of cancer mortality in women, with doxorubicin playing an important role in chemotherapy (CT) regimens used in this setting but limited by cardiotoxicity. Liposomal encapsulated doxorubicin was designed to minimize healthy tissue distribution by altering pharmacokinetics thus reducing cardiotoxicity while preserving antitumor efficacy. The current study was developed to assess the pathologic complete response (pCR), response rate and safety of treatment with taxotere (T), non-pegylated liposomal doxorubicin (M) and cyclophosphamide (C) in previously untreated patients with BC.

Methods: Patients (pts) with HER2-negative BC receiving 1st-line CT were eligible. On Day 1 of each 21-day cycle for a total of 4 cycles, pts received T 75 mg/m² IV, followed sequentially by M 60 mg/m² and C 600 mg/m² IV every 3 wks for 4 cycles. Breast conserving surgery (BCS) was considered if the response was deemed satisfactory, otherwise mastectomy was performed and pCR was evaluated. Pts must have had LVEF ≥50% at the time of enrolment and had regular cardiac evaluation throughout the study (MUGA or ECHO).

Results: A total of 74 pts (50 stages II and 24 IIIA) over a 30-month period in six centres were accrued with clinical/pathological responses available for 51. The median age was 46 yrs (range 23-75). Histology: lobular 7/ductal 67. Grade I/II/III: 6/27/30. ER+/PR+: 43; ER+/PR-: 13. Triple negative: 18. 50 pts had pre-op CT cycles and were subjected to surgery. No clinically significant change in LVEF was noticed (Pre-CT LVEF 68.5% [CI95%: 66.7-70.4]; post-CT LVEF 68.9% [CI95%: 66.0-71.7]).

On an intent to treat analysis, an objective clinical response was observed for 38 pts: complete (12%) and partial (63%). In addition, 12 pts (23%) demonstrated stable disease and only one progressed (2%). The proportion of patients requiring mastectomy and BCS were 14 (28%) and 36 (72%), respectively. On 33 pts with an axillary dissection, 25 (76%) had involved nodes. After pathological review, 18/50 pts (36%) were devoid of any tumor cells in both breast and axillary dissection, 25 (76%) had involved nodes. On 33 pts with an axillary dissection, 25 (76%) had involved nodes. After pathological review, 18/50 pts (36%) were devoid of any tumor cells in both breast and lymph nodes following CT were 3 (IQR 2-6).

All patients were included for toxicity assessment. Grade 3-4 neutropenia and febrile neutropenia was seen in 23% and 10% pts, respectively. Principal non-hematologic grade 3-4 toxicities included allergic reaction/hypersensitivity in 6% pts, and asthenia, diarrhoea and vomiting in 1%. No pts developed symptomatic CHF. Ten pts discontinued treatment: 5 due to taxane-hypersensitivity reaction, 3 due to disease progression and 2 due to protocol violation.

Conclusions: This multi-centre phase II trial clearly demonstrates significant activity (pCR 36%) for neoadjuvant taxane sequentially followed by non-pegylated liposomal doxorubicin and cyclophosphamide regimen in a HER-2 negative BC population. Overall the treatment regimen was well tolerated. Sequential T→MC is safe and not associated with changes in LVEF. An updated evaluation of efficacy and toxicity profile analysis will be presented.
P3-14-16
Molecular Phenotype and the Use of HER-2 Targeted Agents Influence the Accuracy of Breast MRI after Neoadjuvant Chemotherapy.

Moon H-G, Han W, Noh D-Y, Ahn S, Kim J, Shin H, Min J. Seoul National University College of Medicine; Dankuk University Hospital

Background: Improved understanding of factors affecting the accuracy of breast magnetic resonance imaging (MRI) after neoadjuvant systemic therapy (NST) can lead to more tailored use of MRI in deciding surgical extent after NST.

Materials and Methods: We analyzed the imaging and clinicopathologic data of 463 patients who underwent NST. We aimed to investigate whether the molecular subtypes, as well as the use of targeted therapies, were associated with changes in the accuracy of MRI predicting residual tumor extent.

Results: The accuracy of MRI predicting the residual tumor extent was most accurate in triple negative breast cancer and was least accurate in Luminal A subtype (Pearson’s correlation coefficient of 0.754 and 0.531, respectively). Multivariate analysis suggested estrogen receptor status as an independent factor influencing the MRI accuracy. In HER2-amplified tumors, the use of HER2-targeted agents was associated with less accurate MRI prediction. Dual HER2-blockade by using trastuzumab and pertuzumab resulted in lowest MRI accuracy among the patients treated with HER2-targeted agents.

Conclusion: The accuracy of MRI in predicting residual tumor extent was lowest in ER positive tumors treated with NST. In HER2 positive tumors, the use of HER2-targeted agents resulted in less accurate MRI after NST.

P3-14-17
Paclitaxel, Carboplatin, and Trastuzumab in a Neoadjuvant Regimen for HER2-Positive Breast Cancer: The TRAIN Study.

Sonke GS, Mandjes IA, Holtkamp M, Schot M, Oosterkamp HM, Wesseling J, Vranken Peeters M-JT, Rodenhuis S, Linn SC. Netherlands Cancer Institute, Amsterdam, Netherlands

Background: Treatment with trastuzumab is highly active in HER2 positive breast cancer, although cardiotoxicity is a well known side effect. The cardiotoxicity of trastuzumab may be aggravated by combined treatment with anthracyclines. Consequently, treatment with trastuzumab is often delayed pending the administration of anthracyclines. Both in vitro and in vivo data suggest that trastuzumab synergizes with a range of chemotherapeutic drugs, including taxoid drugs and carboplatin. In addition, prolonged pre-operative treatment leads to higher pathologic complete remission (pCR) rates. We report the results of a fase 2 trial integrating trastuzumab at the start of a non-anthracycline containing weekly paclitaxel-carboplatin based neo-adjuvant chemotherapy regimen in HER2-positive breast cancer.

Patients and methods: One-hundred patients with stage II or III breast cancer, including inflammatory disease, with HER2 overexpression (immunohistochemistry and/or in situ hybridization) were treated with 24 weekly administrations of paclitaxel 70 mg/m2, carboplatin AUC=3 mg.ml-1.min, and trastuzumab 2 mg/kg (loading dose 4 mg/kg). In cycles 7, 8, 15, 16, 23, and 24 only trastuzumab was given. The primary endpoint was pathologic complete response (pCR) in both breast and axilla. The trial was preceded by an initial pilot cohort of 55 patients treated with the same regimen.

Results: Final efficacy and safety results of all patients included in the phase 2 trial will be reported at the meeting. In the pilot cohort of 55 similarly treated patients, 33% had stage II disease, 69% was clinically node positive, and 49% was ER and PgR negative. Twenty-four patients (41%) had a pCR in breast and lymph nodes. pCR in ER negative patients was 67%, pCR in ER positive patients was 21%. The most commonly reported grade 3/4 toxicities were neutropenia (30%) and thrombocytopenia (27%). Dose reduction was required in 24% of the patients. Grade 3/4 left ventricular systolic dysfunction was not observed.

Conclusion: A weekly carboplatin-paclitaxel-trastuzumab neoadjuvant regimen is highly active in HER2 positive breast cancer with a good safety profile. A subsequent multicenter phase 3 trial will compare this regimen to a similar 16 week regimen preceded by 4 cycles of anthracyclines plus cyclophosphamide.

The study protocol was developed at the joint ECCO-AACR-EORTC-ESMO Workshop on Methods in Clinical Cancer Research in Flims, Switzerland.

P3-14-18
Primary Tumor Response to Neoadjuvant Chemotherapy Is Significantly Associated with Nodal Pathological Complete Response in Breast Cancer Patients with Cytologically Proven Axillary Node Metastasis.

Hwang H, Park S, Lee JS, Kim S-I, Park B-W. Yonsei University College of Medicine, Seoul, Korea

Background

Axillary node status is the most significant factor for the prediction of outcome in breast cancer patients whether they received neoadjuvant chemotherapy or not. However, a reliable method to predict nodal response to neoadjuvant chemotherapy is not established yet. The aim of this study was to identify the predictive factors of axillary pathological complete response (pCR) after neoadjuvant chemotherapy in breast cancer patients with cytologically proven axillary lymph node metastases.

Patients and methods

A total of 123 patients with axillary metastases confirmed by ultrasound-guided fine-needle aspiration biopsy were subsequently treated with four cycles of anthracycline (60mg/m2) plus cyclophosphamide (600mg/m2) followed by doxetaxel (75-100mg/m2) between August 2008 and March 2011. After neoadjuvant chemotherapy, all patients underwent a definite breast surgery with complete axillary lymph node dissection. Clinicopathological parameters were evaluated using a chi-square test and logistic regression model in association with node pCR.

Results

The mean age at diagnosis was 47.9 years in all patients. Eighty-one (65.9%) patients were preoperative clinical T2 stage, 74 (60.2%) showed estrogen receptor (ER)-positivity, and 36 (29.3%) demonstrated HER2-positivity. A pCR of the axilla and breast was determined in 56 (46%) and 40 (31.7%) patients, respectively. Breast pCR rate is significantly associated with age≤50 at diagnosis, ER(-),PR(-) and higher Ki-67 proliferation. Axillary pCR rate is significantly associated with age at diagnosis of 50 or less, smaller primary tumor size at diagnosis, tumor responsiveness to neoadjuvant chemotherapy, estrogen hormone receptors-negativity, higher Ki-67 proliferative index at diagnosis, and subtypes of HER2-enriched or triple negative breast cancer. When these factors entered logistic regression model, tumor response( SD+PD vs CR+PR: OR 8.603, P=0.007), Ki67((-) vs (+): OR 7.157, p=0.009), ER status((+) vs (-): OR 4.943, P=0.036), pre-operation tumor size(>2cm vs ≤2cm : OR 5.116, p=0.044), HER2 status( (-) vs (+) : OR 5.029, p=0.046) remained to be significant for the prediction of axillary pCR, favorable nodal response.
Conclusion
Our study suggests that primary tumor response to neoadjuvant chemotherapy is significantly associated with favorable nodal response. The factors of tumor and nodal response were similar. There are many issues remained to be determined yet.

P3-14-19
Impact of Chemotherapy-Induced Amenorrhea on Response to Neoadjuvant Chemotherapy in Breast Cancer.
Ahn SK, Moon HG, Kim JS, You JM, Shin HC, Han W, Noh D-Y. Seoul National University Hospital

BACKGROUND: Although chemotherapy and ovarian ablation independently improve the outcome of breast cancer, there is controversy about the benefit of chemotherapy-induced amenorrhea (CIA) in breast cancer. We investigated impact of CIA on response to neoadjuvant chemotherapy in breast cancer patients.

METHODS: We reviewed the records of 198 premenopausal patients with breast cancer treated with neoadjuvant chemotherapy between January 2005 and December 2010. Chemotherapy-induced amenorrhea (CIA) was defined as serum FSH level ≥40 IU/L after completion of all scheduled neoadjuvant chemotherapy and prior to definitive surgery.

RESULTS: Among 198 breast cancer patients, 132 pts (66.7%) developed CIA after neoadjuvant chemotherapy. 156 pts (78%) underwent DA chemotherapy. The age of CIA patients was older than non-CIA patients (41.5±5.55 years vs. 38.27± 6.86 years, p=0.001). The incidence of CIA after neoadjuvant chemotherapy was significantly higher in responder group (responder vs. nonresponder: 87 pts (74.4%) vs. 45 pts (55.6%); p=0.006). Additionally, FSH level after all scheduled neoadjuvant chemotherapy was significantly higher in responder group (FSH 56.41±32.41 IU/L vs. 45.76±30.31 IU/L; p=0.021). In univariate analysis, CIA (p=0.006) and total number of chemotherapy cycle regardless of chemotherapy regimen (p=0.04) were significantly associated with tumor response. CIA was only independent factor for tumor response after neoadjuvant chemotherapy on multivariate analysis (p=0.012).

CONCLUSION: CIA after neoadjuvant chemotherapy was significantly associated with response to neoadjuvant chemotherapy in locally advanced breast cancer.

P3-14-20
Concomitant Taxane-Anthracyclin Regimen for Neoadjuvant Chemotherapy of Primary Breast Cancer: Experience from a Cohort of 223 Patients Treated in a Single Institution.
Coguan E, Guizard A-V, Heutte N, Bor C, Allouache D, Delcambre C, Switser O, Guillot J-M, Leither N, Delozier T, Levy C. François Baclesse Cancer Center, Caen, France

BACKGROUND: Although chemotherapy and ovarian ablation independently improve the outcome of breast cancer, there is controversy about the benefit of chemotherapy-induced amenorrhea (CIA) in breast cancer. We investigated impact of CIA on response to neoadjuvant chemotherapy in breast cancer patients.

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CONCLUSION: CIA after neoadjuvant chemotherapy was significantly associated with response to neoadjuvant chemotherapy in locally advanced breast cancer.

P3-14-21
Neoadjuvant Therapy Response, Subtype and BRCA Status in an Underserved Population.

BACKGROUND: Preoperative (neoadjuvant) chemotherapy is typically used for larger operable breast cancer cases, and the degree of pathological response correlates with long term outcome. Therapeutic response also depends on biological and molecular subtype and is increasingly studied in the research setting to identify prognostic biomarkers and potential therapeutic targets. Little is known about the interactions of neoadjuvant response with biomarker subtypes and genetic predisposition in underserved and minority populations.

METHODS: IRB approval was obtained to capture demographic, clinicopathological and genetic testing data on patients diagnosed with invasive breast cancer and treated with preoperative chemotherapy and definitive surgery between 2005 and 2010 at Los Angeles County Medical Center, which serves a primarily Hispanic and indigent population. Treatment followed NCCN guidelines with the exception that not all patients with HER2+ disease received trastuzumab. Genetic counseling and testing has been available at this center since 2007. Pathological complete response (pCR) was defined as no...
residual invasive disease in breast or nodes. Chi-square or Fisher’s Exact test was used to examine associations between pCR and clinical factors, and logistic regression analyses were applied to assess each variable’s contribution to pCR.

Results: Among 104 patients, of whom 79% were Hispanic, the overall pCR rate was 27%. Significantly higher pCR rates were seen in age ≥50, clinical N0, HER2+, triple negative, and lumpectomy cases. No differences in pCR rate was seen in Hispanics vs. others, Grade III vs. I and II or in the 9 BRC4A mutations carriers among 45 tested compared to no mutation or those not tested. Of the 43 patients with HER2+ disease, the pCR rate was higher in the 32 patients who received trastuzumab (pCR 50.0 vs. 27.3%). Subset pCR rates and odds ratios (OR) of achieving pCR are shown below:

<table>
<thead>
<tr>
<th>pCR Rates and Odds Ratios</th>
<th>pCR (%).</th>
<th>Odds Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hispanic</td>
<td>36/27.8</td>
<td>Not operated</td>
</tr>
<tr>
<td>Age ≥ 50</td>
<td>43/37.2</td>
<td>Other T2</td>
</tr>
<tr>
<td>T1/T2</td>
<td>25/40.0</td>
<td>Ref</td>
</tr>
<tr>
<td>Not operated</td>
<td>78/21.8</td>
<td>0.38</td>
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<tr>
<td>Grade II or I</td>
<td>20/25.3</td>
<td>Ref</td>
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<td>Grade III</td>
<td>74/28.4</td>
<td>1.3</td>
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<td>Mastectomy</td>
<td>88/20.5</td>
<td>Ref</td>
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<tr>
<td>Lumpectomy</td>
<td>16/62.5</td>
<td>8.5</td>
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<tr>
<td>HER2-neg, ER or PR+</td>
<td>35/8.6</td>
<td>Ref</td>
</tr>
<tr>
<td>Triple Negative</td>
<td>26/23.1</td>
<td>1.2</td>
</tr>
<tr>
<td>HER2+</td>
<td>43/44.2</td>
<td>8.4</td>
</tr>
<tr>
<td>Age &lt; 50</td>
<td>61/39.7</td>
<td>Ref</td>
</tr>
<tr>
<td>Age ≥ 50</td>
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<td>Ref</td>
</tr>
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<td>Hispanic</td>
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</tr>
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<td>Non Hispanic</td>
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<td>BRCA Normal</td>
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<td>N0</td>
<td>26/42.3</td>
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<tr>
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</tr>
<tr>
<td>Total HR+</td>
<td>21/42.3</td>
<td>Ref</td>
</tr>
<tr>
<td>Total</td>
<td>42/42.3</td>
<td>Ref</td>
</tr>
</tbody>
</table>

Conclusions: In this underserved cohort, with 43% undergoing genetic testing, significantly higher pCR rates were seen in HER2+ and triple negative and lumpectomy cases, with a trend seen in older patients and smaller tumors. There was an unexpected trend of lower pCR rate seen in BRC4A mutation carriers (pCR OR 0.33), albeit with small numbers. No differences were seen in Hispanic cases compared to other ethnicities. Further tissue analyses are planned to examine established and novel markers and to define exploratory markers that could be used for decision-making and target discovery in larger datasets within this population.

Results: 44 women with a mean age of 74.9 (70-93) years were included. Most tumors were ductal (IDA 88.6%), of grade 3 (79.5%) and hormone-receptor (HR) negative (61.4%). Among the 21 tumors (47.7%) that were HER2+ (immunohistochemistry (IHC) 3+ or 2+ and FISH+), all but 3 received neoadjuvant Trastuzumab. Only 4 patients had no surgery (clinical decision or personal choice) while the remaining 40 patients underwent either breast-conserving surgery (17.5%) or mastectomy (82.5%), 23 patients (52.3%) had pathological partial response, 14 patients (31.8%) pCR and 3 patients no response. pCR was more frequently achieved in HR-negative (44.4%) than in HR- positive patients (11.8%). In both groups HER2 positive tumors had better pathological response than HER2 negative tumors. After a mean follow-up of 41 months (5-120) 29 patients (65.9%) had no evidence of disease, 6 patients (13.6%) had (loco)regional recurrence, 12 patients (27.2%) developed metastatic disease and 6 patients (13.6%) died because of breast cancer. Relapse was more frequently seen in patients with a pPR (47.8%) versus patients with a pCR (14.3%).

Table 1: Pathological response to NACT

<table>
<thead>
<tr>
<th>pCR Rates and Odds Ratios</th>
<th>pCR (%)</th>
<th>Univariate</th>
<th>Multivariate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hispanic</td>
<td>36/27.8</td>
<td>Not operated</td>
<td>0.38</td>
</tr>
<tr>
<td>Age ≥ 50</td>
<td>43/37.2</td>
<td>Ref</td>
<td>Ref</td>
</tr>
<tr>
<td>Hispanic</td>
<td>22/27.3</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>Non Hispanic</td>
<td>22/27.3</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>BRCA Normal</td>
<td>16/27.8</td>
<td>Ref</td>
<td>N/A</td>
</tr>
<tr>
<td>BRCA Mutation</td>
<td>9/11.1</td>
<td>0.38</td>
<td>N/A</td>
</tr>
<tr>
<td>N0</td>
<td>26/42.3</td>
<td>Ref</td>
<td>Ref</td>
</tr>
<tr>
<td>Other</td>
<td>21/42.3</td>
<td>Ref</td>
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<tr>
<td>Total HR-</td>
<td>21/42.3</td>
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</tr>
<tr>
<td>Total</td>
<td>42/42.3</td>
<td>Ref</td>
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</tr>
</tbody>
</table>

P3-14-23 Response to Neoadjuvant Chemotherapy and Survival in Japanese Patients with Triple-Negative Breast Cancer.
Ohtani S, Kochi M, Ito M, Takada S, Matsuura H, Higaki K. Hiroshima City Hospital, Hiroshima, Japan

Background: Triple-negative breast cancer (TNBC) is defined by the lack of estrogen receptor (ER), progesterone receptor (PgR), and human epidermal growth factor receptor 2 (HER2) expression. In this study, we compared response to neoadjuvant chemotherapy and survival between Japanese patients with TNBC and non-TNBC.

Patients and Methods: Analysis of a prospectively collected clinical database was performed. We analyzed 335 Japanese patients who received neoadjuvant chemotherapy at Hiroshima City Hospital for stage I-III breast cancer from 2002-2010. Clinical and pathological parameters, pathological complete response (pCR) rates, survival measurements, and organ specific relapse rates were compared between with TNBC and non-TNBC.

Results: 21.1% had TNBC. Patients with TNBC compared with non-TNBC had not significantly higher pCR rate (28% v 19%; p=0.09), similar disease progression-free survival (DFS) rate (p=0.59), but decreased overall survival (OS) rate (p=0.02). TNBC was associated with increased risk for visceral metastasis (p<0.0001) and shorter postrecurrence survival (p=0.017). If pCR was achieved, patients with TNBC and non-TNBC had similar survival. In contrast, patients with residual disease...
P3-14-24
A Phase II Trial of TS-1 and Docetaxel Followed by 5-FU/Epirubicin/Cyclophosphamide (FEC) as Preoperative Treatment in Women with Stage II/III Breast Cancer.

Hayashi T, Jinno H, Sakata M, Takahashi M, Kitagawa Y. Keio University, School of Medicine, Tokyo, Japan

Background: TS-1 is an oral anticancer drug, composed of tegafur, gimestat and otastat potassium in a molar ratio of 1:0.4:1, based on the biochemical modulation of 5-Fuorouracil. In vivo xenograft model showed a synergistic effect of combination chemotherapy with TS-1 and docetaxel through down-regulation of dihydropyrimidine dehydrogenase (DPD). (Suto et al, Oncology Reports 2006) The sequential combination of anthracycline and taxane is a standard of care in the preoperative setting, as well as the adjuvant setting. This study’s purpose was to determine the efficacy of TS-1 and docetaxel followed by fluorouracil/epirubicin/cyclophosphamide (FEC) in the preoperative setting.

Patients and Methods: Patients with stage II/III breast cancer received 4 cycles of TS-1 (TS-1 80 mg/m² on days 1-14 and docetaxel 40 mg/m² on day 1 every 3 weeks), followed by 4 cycles of FEC (fluorouracil 500 mg/m², epirubicin 100 mg/m², cyclophosphamide 500 mg/m² on day 1 every 3 weeks). The tumor objective response was assessed by both caliper and ultrasound. Primary endpoint was the pathological complete response (pCR) rate. Secondary endpoints were the clinical response rate, adverse drug reactions, and the breast conservation rate.

Results: From November 2009 to December 2010, 39 patients were enrolled and 38 patients were assessable for clinical and pathologic responses. The overall response rate (caliper) was 81.5% and ultrasound response rate was 76.3%. The pCR rate was 13.2% (5/38).

The breast-conserving rate in patients whose tumor size was 3 cm or smaller was 92.9%, and 54.5% of patients whose tumor size was larger than 3 cm underwent breast-conserving surgery. The ratio of Ki-67 positive cell and progesterone receptor (PgR) status were significantly correlated with pCR. Grade 4 neutropenia was observed in a patient (2.6%) at the first cycle. The most common grade 3/4 non-hematologic adverse event was hand-foot syndrome, observed in a patient (2.6%) at the first cycle. The most common grade 3/4 non-hematologic adverse event was hand-foot syndrome, observed in a patient (2.6%) at the first cycle. The most common grade 3/4 non-hematologic adverse event was hand-foot syndrome, observed in a patient (2.6%) at the first cycle. The most common grade 3/4 non-hematologic adverse event was hand-foot syndrome, observed in a patient (2.6%) at the first cycle. The most common grade 3/4 non-hematologic adverse event was hand-foot syndrome, observed in a patient (2.6%) at the first cycle. The most common grade 3/4 non-hematologic adverse event was hand-foot syndrome, observed in a patient (2.6%) at the first cycle. The most common grade 3/4 non-hematologic adverse event was hand-foot syndrome, observed in a patient (2.6%) at the first cycle. The most common grade 3/4 non-hematologic adverse event was hand-foot syndrome, observed in a patient (2.6%) at the first cycle.

Conclusions: These results indicate that the sequential combination of TS-1 and docetaxel followed by FEC is a well-tolerated, effective neoadjuvant treatment for stage II/III breast cancer.

P3-14-25
Neoadjuvant Trastuzumab and Paclitaxel Combination Induces a High Rate of Pathological Complete Responses in Locally Advanced Breast Cancer by Exploiting Host Antitumor Immunity.


Background: A phase II study was performed to assess the role of patients’ immune profile in the activity of a trastuzumab-based, non anthracycline containing neoadjuvant chemotherapy (NC) regimen in locally advanced breast cancer (BC) patients (pts).

Methods: Newly diagnosed T2-T3 HER2+ BC pts received a 12 week NC with weekly paclitaxel and trastuzumab, which was discontinued after further 12 weeks before surgery. The % of patients who showed only one year treatment ± hormonal therapy in case of NC, trastuzumab was discontinued and anthracyclines were planned. Blood samples were collected at diagnosis and every 3 months for 1 year then yearly. The % of NK cells, T cells and Treg cells was evaluated by flow cytometry, and serum levels of 9 cytokines were accessed by SearchLight multiplex array technology. Circulating CD8+ T cells specific for a broad spectrum of tumor-associated antigens (Her2, muc-1, mammaglobulin-A, trag-3, survivin, bcl-xL) were enumerated by IFN-γ ELispot and in vitro T-mediated, antibody-dependent cell cytotoxicity (ADCC) was assessed using patients’ PBMCs.

Results: From July 2006, 34 pts were enrolled, median age 46 yrs (range 30-70). Overall objective clinical response rate was 91%, with 53% of pathological complete responses (pCR) and 38% of partial responses. At a median follow-up of 27 months, 4 relapses were noted (1 brain, 1 local breast, 2 bone). No cardiotoxicity occurred. At diagnosis, pts showed an immune profile similar to that of healthy women, whereas higher numbers of Treg cells (p=0.02), lower T cell numbers (p<0.01) and lower amounts of serum IL-2, IL-6, and IL-8 were found in a concomitant control group of untreated HER2- pts. Moreover, spontaneous CD8+ T cells specific for all 13 HLA-A*0201 epitopes were observed in the whole series, with numbers usually higher than those observed in HER2- cases. Notably, patients with pCR, unlike those showing partial responses, retained high numbers of epitope-specific CD8+ T cells throughout the NC treatment, particularly versus survivin and mammaglobulin-A epitopes. Moreover, pts achieving pCR showed at diagnosis a significantly higher efficiency of trastuzumab-mediated ADCC compared to pts with partial responses (p=0.05). The treatment also induced a progressive increase in the number of NK cells and in the efficiency of trastuzumab-mediated ADCC.

Conclusions: NC with paclitaxel and trastuzumab induces high rates of pCR with no cardiotoxicity. This clinical efficacy is favoured by the retained immunological proficiency of HER2+ pts, who may benefit from the immunological synergism between the two drugs. In fact, the increased number and activation of NK cells promoted by paclitaxel likely favour NK-dependent ADCC responses, known as one of trastuzumab’s main mechanisms of action. The possible role of trastuzumab-mediated ADCC in preserving event-free survival and the involvement of paclitaxel in inducing NK cells’ NF-kB nuclear translocation are under evaluation.
P3-14-26
The Effect of Biologic Subtype in Patients Treated with Neoadjuvant Chemotherapy: A UAB Experience.
Keene KS, De Los Santos JF, Meredith R, Hinton B, Li Y, Krontiras H, Bland K, Carpenter JT, Forero A. University of Alabama at Birmingham, Birmingham, AL

Purpose: Previous studies have suggested that the pre-treatment clinical stage drives loco-regional recurrence (LRR), distant metastasis (DM) and survival in patients treated with neoadjuvant chemotherapy. This retrospective analysis was performed to look at the effect of biologic subtype on patient outcomes. Methods: Between 1999 and 2005, 115 patients treated with neoadjuvant chemotherapy, surgery, and +/-radiation therapy at UAB were identified. Patient, tumor, and treatment characteristics were recorded. Pathologic complete response was defined as resolution of both invasive disease and DCIS in both the primary and nodal disease. Survival was measured using the Kaplan Meier statistics. Univariate and multivariate analyzes of covariates associated with LRR, DM, progression-free (PFS) and overall survival (OS) were performed. Results: The mean age was 49 years, with a mean follow-up of 5.8 years. Subtype distribution was as follows: 52 luminal A, 17 luminal B, 36 triple negative, 9 Her2+ and one patient with an unknown biologic subtype. Distribution of clinical stage was as follows: 40 IIA, 34 IIB, 26 IIIA, 10 IIIB, and 5 IIEC. Tumors were down-staged following neoadjuvant therapy as follows: 18: pCR, 6: residual DCIS, 17: I, 38: IIA, 11: IIB, 13: IIIA, 5: IIIB, and 7: IIIEC. Pre-treatment clinical stage did not significantly influence LRR, DM or progression-free and overall survival; however, final pathologic T, N and group stage were associated with both progression free, p=0.003, 0.011, 0.005 and overall survival, p=0.02, 0.037, and 0.009. Complete resolution of tumor by mammographic or MR imaging to neoadjuvant chemotherapy, was associated with an increased overall survival, p=0.0025. Univariate analysis did not show a significant effect of biologic subtype, age, grade, use of radiation therapy or anti-hormonal therapy. Discussion: In this retrospective series, response to chemotherapy and the final pathologic stage, representing the volume of residual disease, were important predictors of survival. Further study to determine factors predictive of chemotherapy response is needed.

P3-14-27
Pegylated Liposomal Doxorubicin (PLD) as Primary Treatment in Estrogen Receptor (ER) and HER2 Poor Breast Cancer and Risk of Developing Cardiotoxicity or Elderly Patients (pt). Results from the Phase II CAPRICE Study.

Background: Combinations of doxorubicin (DX) and taxanes are considered the standard treatment in high risk breast cancer, but classical DX is not commonly used in elderly pt or in those with risk of developing cardiotoxicity. PLD (Caelyx/Doxil™) is associated with less cardiotoxicity. We present a multicentric phase II trial conducted by SOLTI group. A combination of PLD and cyclophosphamide (CP) followed by paclitaxel (PTX) was tested as primary chemotherapy (CT) in pts with stage II-IIIB breast cancer with poor estrogen receptor positivity (< 50% positive nuclei) and at least one risk factor of developing cardiotoxicity. Our aim was to demonstrate a pathologic complete response (pCR) rate similar of prior DX studies without cardiotoxicity. Method: PLD 35 mg/m^2 + CP 600 mg/m^2 were administered every 4 weeks (w) for 4 cycles followed by PTX 80 mg/m² every w for 12 w. Left ventricular ejection fraction (LVEF), ECG and cardiac questionnaire were performed at baseline and 8, 16, 28, 40 w thereafter. The primary end point was pCR. Secondary endpoints included: cardiac safety, radiological response rate (RRR), breast conserving surgery (BCS) rate, toxicity and overall survival at 5 years. Results: 50 pt were included. Median age: 73 (35-84), 42 pt > 65 years old; 32 pt (64%) suffer from hypertension and 7 had prior cardiac disease. Histological grade III: 36 pts (72%); stage II/III: 24/26 pt respectively; only 13 pts (26%) were candidate for BCS at diagnosis. 46 pt (92%) underwent surgery: BCS 27 (58.6%) and mastectomy 19 (41.3%); 4 pt did not proceed to surgery due to serious adverse events. In an intent-to-treat analysis pCR rate in breast was 32% (95% CI 19.5-46.7%), 22% (95% CI 10.5-33.4%) in breast and nodes. Among triple negative pt (N=48) pCR was 33.3%. Two of seven T4d pt (28.7%) achieved pCR. No significant decrease in LVEF was seen: Mean baseline LVEF was 66.6% (52-86), 66.7 (51-88) after 16w, 62.2 (48-75) after 28w and 64.7 (50-74) after 40 w. Other toxicities: edema 10%, alopecia 10%, neutropenia 10%, stomatitis 8%, neurotoxicity 8%, skin reactions 6%, Hand-Foot Syndrome 6%. Grade III-V toxicities were reported in 10 patients (20%): cardiovascular 3, diarrhea 2, rash 2, lung 2 and neutropenia 1. Three non-cancer deaths occurred: 1 sudden death in a 82 year’s old pt one month after surgery, 1 due to hemorrhagic stroke > 30 days after completing CT pt with prior cerebrovascular disease, and another 84 year’s old due to non-neutropenic pneumonia during CT. Conclusions: This schedule of primary chemotherapy achieves a pCR rate similar to the standard treatment and is feasible in a group of patients for whom DX was contraindicated. This strategy enables to double the BCS rate. Toxicity was acceptable in a very fragile cohort of patients.

P3-14-28
ANZ 0502 NeoGem: A Phase II Trial Evaluating the Efficacy and Safety of Epirubicin and Cyclophosphamide Followed by Docetaxel with Gemcitabine (+ Trastuzumab IF HER2 Positive) as Neoadjuvant Chemotherapy for Women with Large Operable or Locally Advanced Breast Carcinoma.
McCarthy N, Boyle F, Bull J, Leong E, Simpson A, Kannourakis G, Gebski V, Forbes JF, Wilcken N, Lindsay DF, Badger HD, Royal Brisbane and Women's Hospital, Brisbane, Queensland, Australia; The Mater Hospital, Sydney, New South Wales, Australia; Wellington Hospital, Wellington, New Zealand; Ballarat Oncology and Haematology Service, Ballarat, Victoria, Australia; NHMRC Clinical Trials Centre, Sydney, New South Wales, Australia; University of Newcastle, Newcastle, New South Wales, Australia; Calvary Mater Newcastle, Newcastle, New South Wales, Australia; Westmead Hospital, Sydney, New South Wales, Australia; Australian New Zealand Breast Cancer Trials Group, Newcastle, New South Wales, Australia

Background: Neoadjuvant chemotherapy may provide an early indication of treatment effect and pathologic complete response (pCR) rate is a surrogate measure of disease-free and overall survival. Anthracyclines remain an important component of chemotherapy regimens for breast cancer (BC), adding a taxane conveys additional survival benefit. Gemcitabine (G) has established safety and efficacy in metastatic breast cancer (MBC) and combining G with docetaxel...
(D) shows preclinical synergy but not overlapping toxicities. In MBC, efficacy of trastuzumab (T) combined with single agent taxanes and G has been demonstrated for tumours that over-express human epidermal growth factor receptor 2 (HER2+). NeoGem aimed to evaluate the efficacy and safety of neoadjuvant epirubicin (E) and cyclophosphamide (C), followed by D and G +/- T (depending on HER2 status) in women with large operable or locally advanced BC.

**Methods:** Eligible patients (pts), ≥18 years, had unilateral, operable (at presentation) T2 (≥3 cm), T3-4, N0-1, M0 primary BC, no prior chemotherapy or hormonal therapy and ECOG status 0-2. All pts received E (90mg/m^2^ i.v.) in combination with C (600mg/m^2^ i.v.) on day 1 q 21 for 4 cycles followed by D (75mg/m^2^ i.v.) on day 1 in combination with G (1000mg/m^2^ i.v.) on days 1 and 8 q 21 for 4 cycles. HER2+ pts received T (4mg/kg loading then 2mg/kg i.v.) concurrent with DG on days 1, 8 and 15 q 21 for 4 cycles. HER2+ pts received post-surgical T (6mg/kg) 3 weekly, for a total of one year of T therapy. Using a Simon’s 2 stage trial design, the decision to proceed to stage 2 followed interim analysis of stage 1. Primary endpoint, pCR, was defined as no histologic evidence of invasive cancer in the breast. Secondary endpoint, pCRax, was defined as no histologic evidence of invasive cancer in the breast and axilla. EC followed by DG/DGT was expected to achieve a pCR rate of 35% in HER2 negative (HER2-) pts and 40% in HER2+, with the lowest limit of therapeutic efficacy being a pCR rate of 22% (HER2-) and 24% (HER2+). Hence 84 HER2- and 63 HER2+ pts were needed to detect significant differences in pCR rates (power 80%, 95% level of significance).

**Results:** Over 32 months 81 pts (63 HER2- and 18 HER2+) were enrolled, 78 (96%) (61 HER2- and 17 HER2+) proceeded to surgery. Of 78 pts, 21 (27%) achieved pCR and 19 (24%) achieved pCRax. Of the 61 HER2- pts, 12 (20%) (95% CI: 12%-31%) achieved pCR compared with 9 (53%) (95% CI: 31%-74%) of the 17 HER2+ pts. Planned chemotherapy was completed by 67 pts (83%), 9 pts (11%) discontinued due to adverse events. Thirteen pts (16%) required DG dose reductions compared with 7 (8%) pts during EC; 57 (70%) pts had ≥ grade 3 neutropenia.

**Conclusion:** Efficacy in the HER2- cohort did not reach predetermined levels of significance (interim analysis); HER2+ recruitment proved too slow to continue. High haematological toxicity during DG, particularly neutropenia required use of supportive therapy (GCSF). Despite relatively small patient numbers, 53% pCR in the HER2+ cohort warrants further investigation.

**P3-14-29**

**Neoadjuvant Sunitinib Administered with Weekly Paclitaxel/Carboplatin in Patients with Locally Advanced Triple-Negative Breast Cancer: A Sarah Cannon Research Institute Phase I/II Trial.**


**Background:** Angiogenesis plays a substantial role in breast cancer development as well as in triple negative breast cancer (TNBC). Sunitinib is an inhibitor of the tyrosine kinase receptors for VEGF, platelet-derived growth factor (PDGF), KIT, RET, and fms-like tyrosine kinase receptor-3 (FLT3). As monotherapy in heavily pretreated breast cancer patients (pts), sunitinib demonstrated a response rate of 15% in TNBC (11% of all pts) with stable disease or better in 16% of all pts. The combination of paclitaxel and carboplatin is ideally suited for further exploration as neoadjuvant chemotherapy for TNBC, based on the established preclinical and clinical sensitivity of TNBC to these cytotoxic agents. This open label, phase I/II trial was designed to evaluate the combination of sunitinib plus paclitaxel and carboplatin as neoadjuvant treatment for locally advanced breast cancer. The primary objective for the phase I portion was to determine the maximum tolerated dose (MTD); these results are presented.

**Methods:** Women with histologically confirmed invasive triple-negative adenocarcinoma of the breast, (defined as <10% staining by IHC for ER/PR; IHC 0-1+ or FISH negative for HER2), with no evidence of metastatic disease and normal LVEF were eligible. All pts received sunitinib (days 1-28), paclitaxel (days 1, 8, 15), and carboplatin (day 1) in 28-day treatment cycles x6. Following 6 cycles, pts had definitive surgery. After ≥2 weeks and evidence of adequate wound healing, maintenance sunitinib 25mg PO daily was initiated to complete a total of 52 weeks. Three dose levels were evaluated as shown in the table below:

<table>
<thead>
<tr>
<th>Dose Level</th>
<th>No. of Pts</th>
<th>Paclitaxel (mg/m^2^)</th>
<th>Carboplatin (AUC)</th>
<th>Sunitinib (mg PO)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6</td>
<td>70</td>
<td>5</td>
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</tr>
<tr>
<td>3</td>
<td>3</td>
<td>80</td>
<td>6</td>
<td>25</td>
</tr>
</tbody>
</table>

Doses were escalated in sequential cohorts of pts using standard phase I methodology. MTD was defined as the highest dose level (DL) producing ≤1 dose limiting toxicities (DLTs) in a pt cohort. The MTD identified in the phase I portion of the study will be used in the phase II portion, which will evaluate the efficacy, safety, and tolerability of this combination in pts with locally advanced TNBC.

**Results:** 15 women with TNBC were enrolled between 10/2009 and 2/2011 [median age 53 years (range: 40-78)]. Due to grade 3 neutropenia resulting in the inability to deliver cycle 1 day 15 paclitaxel in the first pt treated at both DLs 1 and 2, these DLs were expanded to 6 pts each. No additional cycle 1 DLTs were noted in the 5 additional pts at either DL. Three pts were accrued to DL 3; there were 2 DLTs noted among these pts (grade 3 febrile neutropenia; grade 3 neutropenia with cycle 2 day 1 treatment delay). However, due to the development of grade 3/4 neutropenia in subsequent cycles in 5 of 6 DL 2 pts, resulting in dose delays and requiring dose reductions, the MTD of this combination was defined as DL 1 (paclitaxel 70mg/m^2^ (Days 1, 8, 15); carboplatin AUC=5 (Day 1); sunitinib 25mg PO daily).

**Conclusions:** The administration of sunitinib with paclitaxel plus carboplatin as neoadjuvant therapy is feasible with neutropenia defining the MTD of this combination. The phase II portion of this study is ongoing.

**P3-14-30**

**Concurrent Celecoxib with FEC Followed by Docetaxel Shows Good Responses and Prognosis in a Neoadjuvant Breast Cancer Study.**

Chow LWC, Tung SY, Ng T-Y, Oh D-Y, Im S-A, Lee M-H, Yip AYS, Toi M, Glück S. Organisation for Oncology and Translational Research, Hong Kong; UNIMED Medical Institute, Hong Kong; Tuen Mun Hospital, Hong Kong; Seoul National University Hospital, Seoul, Korea; Soonchunhyang University Hospital, Seoul, Korea; Kyoto University, Kyoto, Japan; University of Miami Leonard M. Miller School of Medicine, Miami, FL

**BACKGROUND:** Anthracyclines and taxane have been widely used as neo-adjuvant therapy(NAT) for breast cancer. Addition of COX-2 inhibitors might enhance the anti-cancer effect of chemotherapy. This prospective clinical trial studied 5-fluorouracil/Epirubicin/Cyclophosphamide-Docetaxel(FEC-T) with concurrent celecoxib(CXB) as NAT for breast cancer patients(pts). Primary endpoints were pathologic complete response(pCR) and clinical
response (CR), secondary endpoints were safety and breast conservative therapy (BCT) rate. Disease-free survival (DFS) was also determined. METHODS: This study accrued 91 pts among which 87 pts received FEC (F:500mg/m2; E:100mg/m2; C:500mg/m2) followed by T(100mg/m2). In the initial phase, the study randomized 12 and 11 pts to receive 4 cycles of FEC followed by 4 cycles of T with or without concurrent CXB (400mg bid) respectively. Due to possible cardiovascular events from COX-2 inhibitors, the study protocol was revised to a single arm phase II study which recruited another 64 pts who received FEC-T with concurrent CXB at reduced dosage (200mg bid). Primary endpoints were compared between pts with and without CXB using Fisher’s Exact test. All endpoints and survival were evaluated for phase II subjects. RESULTS: Out of 87 pts, 84 invasive breast cancer pts, age ranged 30-62 (mean: 46±6 years) and clinically staged IA (n=48, 57.1%), IIB (n=28, 33.3%) and IIA (n=8, 9.5%), are eligible for comparison between groups (CXB+, n=73 / CXB-, n=11). More CRs were observed in CXB+ than in CXB- (94.5% vs. 72.7%, p=0.044) but pCR did not show statistical difference. In the phase II study, 57/64 pts, including luminal A 35/61 (54%), luminal B 12/21 (57.1%), HER-2 positive 8/14 (57.1%), and triple negative (TPN) 12/30 (40%), completed NAT and definitive surgery. pCR plus near pCR was observed in 18/31 (58%) pts. Excluding TPN subtype, pCR (p=0.761) did not but near pCR (p=0.043) differed among subtypes. Those with HER-2 amplification had higher near pCR than those who did not (6/20 vs. 3/37, p=0.031). Out of 56 pts evaluable for CR, 43 (75.4%) reached complete and partial CR respectively. Over 80% pts received BCT after NAT and that 11/14 (78.6%) pts who initially were unsuitable for BCT with a baseline tumor ≥5cm became eligible after NAT. At a median follow-up of 37 months, 61 pts who completed ≥6 cycles of NAT and surgery were followed and 80% of pts are still disease-free. Pts responding to the NAT has fewer chance of relapse (p=0.03). Neither life-threatening toxicity nor cardiotoxicity was observed. The most commonly observed grade 3/4 adverse events included neutropenia and leukopenia: 39.1% and 20.3% during FEC, and 16.1% and 12.9% during T respectively. Febrile neutropenia was observed in 9.4% pts during FEC, but none during T. Less than 40% of pts required G-CSF support. Other mild toxicities were manageable. CONCLUSIONS: Higher CR was observed in pts receiving neo-adjuvant FEC-T with concurrent CXB which appeared well-tolerated and safe. Rate of BCT is high after the treatment. Pts with HER-2 amplified breast cancer responded better than those who did not. Chance of relapse is significantly reduced for NAT responders. Further investigation of using COX-2 inhibitors with neo-adjuvant chemotherapy is warranted.

P3-14-31
Preoperative Chemotherapy and Bevacizumab for Locally Advanced HER-2 Negative Breast Cancer Followed by Prolonged Postoperative Bevacizumab for Those with Less Than Complete Pathologic Response.
Carpenter JT, Forero A, Falkson CI, Nabell LM, De Los Santos JF, Krontiras H, Bland KD, Li Y. University of Alabama at Birmingham, Birmingham, AL

We studied patients with locally advanced breast cancer (LABC) to evaluate 1) whether adding concurrent bevacizumab to preoperative chemotherapy (CT) would increase the pathologic complete response (pCR) rate, 2) whether a prolonged postoperative course of bevacizumab would prevent or reduce the recurrence rate in patients who did experience pCR after preoperative chemotherapy, and 3) whether use of pegylated liposomal doxorubicin would prove comparably effective to doxorubicin with less toxicity in this setting. From 3/08-12/09 32 patients with HER-2 negative operable (LABC) received sequential preoperative CT with pegylated liposomal doxorubicin 25 mg/M2 every 2 weeks X 3, paclitaxel 175 mg/M2 every 2 weeks X 3, and cyclophosphamide 60 mg/M2 every 2 weeks X 3, all with concurrent bevacizumab 10 mg/kg every 2 weeks. One patient’s disease progressed by the end of CT; 1 patient withdrew after 2 cycles of treatment. All 30 remaining patients had ≥30% shrinkage of tumor at the completion of CT. Left ventricular ejection fractions on the 31 who completed CT were >55% before and after completing CT; no cardiac failure was seen. Grade III toxicities included hypertension >150/90 in 7 and palmar-plantar erythrodysesthesia in 1; other toxicities were grade 1 or II. No proteinuria was seen. Five experienced delayed wound healing after operation. Responses: 9/32 pCR in breast and axillary nodes (2/15 in ER or PR+, 7/17 in ER/PR-), and 3 pCR in breast with ≥1 positive axillary node (2 ER or PR+, 1 ER PR-). Hormonal treatment but no further CT was given after operation. 20/21 patients with residual invasive cancer in breast or nodes started an additional year of bevacizumab 15 mg/kg every 3 weeks; 1 never started bevacizumab due to delayed wound healing- her disease recurred before starting bevacizumab and she died 22 months after diagnosis. All but 6 of these have completed the additional year of bevacizumab (1 withdrew early due to the need for 2 emergency abdominal operations); 1 experienced recurrence in lungs during bevacizumab treatment and subsequently died 24 months after starting treatment (ER+). An additional patient with initial pCR experienced recurrence in brain, then lung 18 months after starting treatment (ER/PR-). With a median followup of 26.5 months since diagnosis, 30 of 32 remain alive, 28 of 32 remain free of recurrence, and 19 of the 20 who started bevacizumab for less than pCR after operation remain free of recurrence. Concurrent bevacizumab did not appear to influence the pCR rate from preoperative CT. The pCR rate observed using pegylated liposomal doxorubicin is comparable to that obtained at UAB with sequential treatment using doxorubicin, but is less toxic. Use of bevacizumab after operation in patients with LABC who do not experience pCR from preoperative chemotherapy may decrease recurrence and appears promising. Further observation in this group and further prospective study will be of interest. Supported by Centocor Ortho Biotech Services and by Genentech.

P3-15-01
An Interim Efficacy Analysis of Neoadjuvant Letrozole in the New Primary Endocrine-Therapy Origination Study (NEOS/N-SAS BC06): A Randomized Study of Adjuvant Endocrine Therapy with or without Chemotherapy for Postmenopausal Breast Cancer Patients Who Responded to Neoadjuvant Letrozole.
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Background: It has not been established if adjuvant chemotherapy is required for patients with intermediate-risk endocrine-responsive postmenopausal breast cancer. The NEOS is a randomized controlled trial to verify the necessity of adjuvant chemotherapy in patients with node-negative, ER-positive, and HER2-negative postmenopausal breast cancer who responded to neoadjuvant endocrine therapy. For
neoadjuvant letrozole (LET), the protocol specified that an interim analysis should be performed to avoid lack of benefit to patients if no therapeutic effects, which were anticipated before the start of the study, were obtained.

Methods: Patients who complied with the eligibility criteria were administered LET preoperatively in weeks 24-28 after primary enrollment. Patients evaluated as complete response (CR), partial response (PR) or stable disease (SD) underwent secondary enrollment and will be divided at random into two arms, an arm given LET for 4.5-5 years after chemotherapy and another arm given only LET for 4.5-5 years. Patients evaluated as progressive disease during LET treatment will receive discretionary treatment. For neoadjuvant LET, the number of patients requiring interim analysis of efficacy was calculated as 140 based on the assumption that the threshold response rate of CR+PR+SD was 75% and its expected response rate was 85% in patients who completed neoadjuvant LET, and that the one-sided level of significance was 5% and the statistical power was 90%. When the hypothesis that the rate of CR+PR+SD was less than 75% was not rejected at a significance level of 5%, the study was discontinued and when it was rejected, the study was continued.

Results: As of June 2011, 140 patients in 39 centers had completed neoadjuvant LET. The median age was 64 years old (range: 50-75) and the median Body Mass Index (BMI) was 23.90 (±3.77). According to MRI or CT scan, CR occurred in 2 (1.4%), PR in 57 (40.7%), and SD in 59 patients (42.1%). The rate of CR+PR+SD among the 140 patients was 84.3% (90% confidence interval: 78.6-88.7). In ultrasonography, 47 patients (33.6%) showed a decrease in the long diameter of 30% or more in a mono-dimensional measurement and 52 patients (37.1%) showed a decrease in volume of 50% or more in a three-dimensional measurement. The neoadjuvant LET effects showed no differences in patients with a BMI of less than 20, 20-25, and more than 25. No serious adverse events were observed.

Discussion: The rate of patients who achieved CR+PR+SD by neoadjuvant LET complied adequately with the continuation criteria for the study. We will present the results for 205 patients who could be evaluated for efficacy and safety of neoadjuvant LET as of May 2011. This study was supported by Public Health Research Foundation. Clinical Trial Registration Information: UMIN (http://www.umin.ac.jp/), Study ID: 000001090

P3-15-02
The Change of Bone Turnover Markers during Neoadjuvant Anastrozole Versus Exemestane: A Randomized Single-Center Study.

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Background
Non-steroidal aromatase inhibitors including anastrozole and letrozole have been reported to increase the rate of bone turnover, accelerate loss of bone, and increase the incidence of fractures. Although steroidal inactivator exemestane has similar inhibitory effect on the aromatization, animal studies have shown a weak but potentially important anabolic effect of exemestane metabolites, which might lead to decreased bone resorption. This randomized trial was conducted to compare the effect of exemestane and anastrozole on bone turnover markers.

Patients and Methods
Fifty-two postmenopausal women with ER-positive, HER2-negative, invasive, nonmetastatic, and operable breast cancer were randomly assigned to neoadjuvant exemestane (25 mg daily) or anastrozole (1 mg daily) for 4 months. The primary endpoint was change in bone turnover markers including resorption markers urinary and serum N-telopeptide (NTX) and the formation markers serum bone alkaline phosphatase (BAP). The secondary endpoint was tumor objective response (OR) assessed by both caliper and ultrasound. Comparisons were also made of breast conservation rate and adverse events.

Results
The changes in serum NTX from baseline to week 16 were not statistically different between anastrozole and exemestane. The changes in urinary NTX from baseline to week 16 were also statistically different between both groups. BAP did not show any significant increase in the exemestane group (10.8%; p=0.21), whereas the increase in BAP from baseline to week 16 in the anastrozole group was 15.0% and marginally significant (p=0.05). There were no significant differences in OR in the intent-to-treat population between patients receiving anastrozole or exemestane (45.8% vs. 39.2%; p=0.63). The OR was similar between the patients with a baseline Ki67 index of >15% and <15% in exemestane group (37.5% vs. 41.2%; p=0.86). In anastrozole group the OR in patients with a baseline Ki67 index of >15% and <15% was 44.4% and 53.8%, respectively (p=0.66). Breast conservation rate was similar between anastrozole and exemestane (62.5% vs. 67.9%; p=0.68). Treatment was well tolerated and much the same for both groups.

Conclusions
There is no significant differences of the change in bone turnover markers between anastrozole and exemestane. These results indicate exemestane had no anabolic effects which would lead to fewer adverse effects on bone.

P3-16-01
Safety Profile and Clinical Activity of Single-Agent BKM120, a Pan-Class I PI3K Inhibitor, for the Treatment of Patients with Metastatic Breast Carcinoma.

Rodon J, Bendell JC, Abdul Razak AR, Homji N, Trandafir L, Quadt C, Graha-Suárez B, Siu LL, Di Tomaso E, Demanse D, Massacesi C, Hirawat S, Burtis III HA, Baslega J. Vall d’Hebron University Hospital, Barcelona, Spain; Sarah Cannon Research Institute, Nashville, TN; Princess Margaret Hospital, Toronto, ON, Canada; Novartis Pharmaceuticals, Florham Park, NJ; Novartis Oncology, Paris, France; Novartis Pharma AG, Basel, Switzerland; Novartis Institutes for BioMedical Research Inc, Cambridge, MA; Massachusetts, Boston, MA

Background
Phosphatidylinositol 3-kinase (PI3K) is critical to cancer cell growth, survival, and metabolism. BKM120 is an oral pan-class I (α, β, γ, δ) PI3K inhibitor that has demonstrated in vitro and in vivo tumor cell growth inhibition in a range of cancer types including breast cancer.

Materials and methods: The Phase I study CBKM120X2101 investigating single-agent daily BKM120 in patients (pts) with advanced solid tumors has been recently completed with the maximum tolerated dose established at 100 mg/day. Here, we report the analysis of metastatic breast carcinoma (MBC) pts enrolled in this study.

Results: Overall, 83 pts have enrolled, 21 of whom have MBC. At the cut-off date of 25th February 2011, 20 MBC pts were evaluable: 1 pt at 80 mg, 1 pt at 150 mg and 18 pts at 100 mg. Patient characteristics were as follows: median age 55 years (range 37–71); performance status ECOG 0/1/2 for 7/12/1 pts, respectively; visceral disease was reported in 16 pts, including liver, 10 pts (50%); lung, 9 pts (45%); and pleura, 5 pts (25%); all pts had ≥3 lines of systemic therapy (3–12). The median time from last treatment and study entry was 4.5-5 years. Patients evaluated as progressive disease during LET and after 4.5-5 years after chemotherapy and another arm given only LET for 4.5-5 years. Patients evaluated as progressive disease during LET treatment will receive discretionary treatment. For neoadjuvant LET, the number of patients requiring interim analysis of efficacy was calculated as 140 based on the assumption that the threshold response rate of CR+PR+SD was 75% and its expected response rate was 85% in patients who completed neoadjuvant LET, and that the one-sided level of significance was 5% and the statistical power was 90%. When the hypothesis that the rate of CR+PR+SD was less than 75% was not rejected at a significance level of 5%, the study was discontinued and when it was rejected, the study was continued.

Results
The changes in serum NTX from baseline to week 16 were not statistically different between anastrozole and exemestane. The changes in urinary NTX from baseline to week 16 were also statistically different between both groups. BAP did not show any significant increase in the exemestane group (10.8%; p=0.21), whereas the increase in BAP from baseline to week 16 in the anastrozole group was 15.0% and marginally significant (p=0.05). There were no significant differences in OR in the intent-to-treat population between patients receiving anastrozole or exemestane (45.8% vs. 39.2%; p=0.63). The OR was similar between the patients with a baseline Ki67 index of >15% and <15% in exemestane group (37.5% vs. 41.2%; p=0.86). In anastrozole group the OR in patients with a baseline Ki67 index of >15% and <15% was 44.4% and 53.8%, respectively (p=0.66). Breast conservation rate was similar between anastrozole and exemestane (62.5% vs. 67.9%; p=0.68). Treatment was well tolerated and much the same for both groups.

Conclusions
There is no significant differences of the change in bone turnover markers between anastrozole and exemestane. These results indicate exemestane had no anabolic effects which would lead to fewer adverse effects on bone.
46 days (29–235). The median duration of BKM120 treatment administered was 7.5 weeks (1.0–96.4). The most frequent grade 3 drug-related adverse events (AEs) were: transaminases increase, 4 pts; psychiatric disorders, 3 pts, consisting of anxiety, affective disorder, and mood alteration (1 pt each); diarrhea, 2 pts; fatigue, 2 pts; and hyperglycemia, 1 pt. The only grade 4 drug-related AE was hyperglycemia, reported in 1 pt at 150 mg. Most AEs were manageable with treatment interruption and dose reductions. Eighteen pts were evaluable for objective tumor response by RECIST. Two pts (11%) exhibited partial responses, which were confirmed in a triple-negative MBC pt, and unconﬁrmed in an ER+ HER2– MBC pt. For these 2 pts, the treatment duration was 29+ (ongoing) and 6 months, respectively. An additional 9 pts (50%) had stable disease, lasting >4 months in 7 pts (35%).

Conclusions: This preliminary analysis showed that BKM120 has single-agent activity in heavily pretreated pts with MBC, and an acceptable safety proﬁle. Molecular proﬁling and updated pharmacokinetic results will be presented at the meeting.

P3-16-02
Targeting Tumor Initiating Cells with siRNA/Nanotherapy in Triple Negative Breast Cancer.
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Residual human breast tumor cells after conventional therapies are enriched in tumor-initiating cells (TIC) characterized by CD44+/CD24−/low lineage with self-renewal capacities. Our gene expression analyses in those cells and in breast cancer cells propagated as mammospheres (MSs) reveal an epithelial-mesenchymal-transition (EMT) signature (400 genes) mainly found in claudin-low molecular subtype human breast tumors. We performed lentiviral shRNA knockdown of that signature in MS. Critical shRNAs were found to decrease the TIC subpopulation. Among those genes, we found STAT3 (signal transducer and activator of transcription 3), NTN4 (netrin 4), and RPL39L (ribosomal protein L39-like). Here, we used a multiscale siRNA delivery system targeting those three genes in order to clarify the effect of silencing those genes over the self-renewal capacity on MSs with claudin-low features. To this end, duplex small interfering RNA (siRNA) against STAT3, NTN4, RPL39L, and scrambled siRNA as control, were introduced into neutral nanoliposomes (dioleoyl phosphatidylcholine, DOPC), and using mesoporous microscale biodegradable silicon particles as carriers. This multimodal siRNA delivery system has been reported as a good approach for sustained gene silencing. Claudin-low-like human breast cancer cell lines (SUM159 and BT549) were plated in 24-well ultra-low attachment plates with mammary epithelial growth medium (MEGM) (5,000 cells/well). Both cell lines were then treated with 1 µg/well/6 wells of silicon particles loaded with DOPC nanoliposomes/siRNA. The primary MSs were allowed to grow for 3 days. MSs were counted by day 3 with a GelCount colony counter (Oxford Optronix, Oxford, UK). Mammosphere-Forming Efficiency (MSFE) was calculated by dividing the number of MSs by the number of seeded cells. In addition, established MSs were serially passaged by dissociation, and single cells were replated on fresh 24-well ultra-low attachment plates to form secondary mammospheres, which were counted after 3 days. One-way ANOVA and Tukey test were performed. A p value less than 0.05 was considered as signiﬁcant. Our results show that silencing STAT3, NTN4, and RPL39L signiﬁcantly reduces the MSFE in both primary (1%, 0.9%, and 1.7% respectively) (Figure 1) and secondary MSs (0.49%, 0.51%, and 0.45% respectively) (Figure 2) when compared to the scrambled control (2.4% and 1% respectively) in BT549 cells. For SUM159 cells, we did not ﬁnd any change in primary MSs for STAT3 (2.2%) and NTN4 (2.1%), even we found a higher percentage of MSFE in those cells treated with RPL39L siRNA (3.9%) when compared to the scrambled control (2.1%) (Figure 3). Nevertheless, a lesser MSFE were observed in those cells treated with STAT3 (0.9%, not signiﬁcant), NTN4 (0.8%) and RPL39L (0.78%) in comparison to control (1.1%) (Figure 4). In conclusion, knocking-down of EMT-related genes (STAT3, NTN4, and RPL39L) decreases signiﬁcantly the self-renewal capacity in mammospheres derived from claudin-low-like human breast tumor cells, being BT549 cells more sensitive than SUM159 cells to that silencing with siRNA loaded in DOPC nanoliposomes into silicon particles as carriers.

Acknowledgments
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P3-16-03
Enhancing Anti-IGF Directed Therapeutics by Co-Targeting Autophagy.
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Autophagy (macautophagy) is a cellular mechanism that maintains homeostasis and cell growth by regulating the turnover of damaged proteins. Autophagy has a critical role in maintaining cellular viability during nutrient starvation and metabolic stress by recycling intracellular proteins for nutrient reallocation. Whether the autophagy pathway utilized by breast cancer cells has a survival mechanism or is a feature of cell death is not fully understood. Since the IGF/insulin signaling maintains cellular growth, nutrient utilization, and survival, increased autophagy or autophagic flux might be induced with IGF-1 inhibitors as a compensatory survival mechanism. In breast cancer cells treated with anti-IGF1R kinase inhibitors, we observed an increase in autophagic flux; similar to levels induced by nutrient deprivation.

Objective: In this study, we examined whether IGF1R inhibitor-induced autophagy is cytoprotective or cytotoxic. If autophagy has a protective role in nutrient-depleted conditions it could reduce the long term efficacy of IGF1R inhibition by making cells refractory to blockade of this signaling system. In this study, we hypothesized that blocking autophagy in combination with anti-IGF1R therapies (antibodies or small molecules) would further enhance growth inhibition in a panel of breast cancer cells types (luminal and basal-like/triple negative).

Results: We initially examined whether autophagy inhibitors (chloroquine or bafilomycin A1) had a differential growth effect on breast cancer cells that varied in their sensitivity to IGF1R stimulated growth. We found that low-to-moderate IGF growth dependent MDA-MB-231 and MDA-MB-435A breast cancer cells were more sensitive to chloroquine alone. However, sensitivity to bafilomycin A1 differed between breast cancer cell lines compared to chloroquine. In conditions of cellular stress when autophagy is normally triggered, we observed an increase in autophagic flux; similar to levels induced by nutrient deprivation.

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with IGF1R inhibitors, but not in IGF1R inhibitor resistant cancers. Additionally, targeting autophagy as a single agent therapy may be more beneficial in triple negative breast cancer compared to other subtypes of breast cancer.

**P3-16-04**  
**A Novel Monoclonal Antibody to Secreted Frizzled Related Protein 2 Inhibits Triple Negative Breast Carcinoma Growth Rate In Vivo.**


**Background:** Secreted frizzled related protein 2 (SFRP2) is a novel angiogenesis factor expressed in the endothelium of a wide variety of human tumors including triple negative breast cancer and angiosarcoma. We previously reported generating a monoclonal antibody against SFRP2 that inhibits endothelial cell and angiosarcoma tube formation in vitro, and decreased tumor volume of the SVR angiosarcoma in vivo. The objectives of these studies were to determine pharmacokinetic (PK) and pharmacodynamic (PD) parameters of the SFRP2 MAb, and evaluate its efficacy in a triple negative breast cancer xenograft.

**Methods:** I-SFRP2 MAb was administered to nude mice i.v. via tail vein injections at 0.4 mg/kg, 4 mg/kg, or 10 mg/kg in mice with or without tumor. Blood organ, and tumor samples were collected at various time points from 5 min to 21 days. Radiolabeled SFRP2 MAb in serum and tissues was determined using a gamma counter. PK parameters were determined based on mean concentration values for 3-5 animals per time point. In vivo efficacy study: MDA-MB-231 human breast cancer xenografts were established in 6-week-old female nude mice. Mice were inoculated with 1 x 10⁶ cells s.c.. Treatment began on day 16 after tumor inoculation when average tumor size was 200 mm³. Animals were randomly assigned (n = 12 per group) to buffer control, SFRP2 MAb 4 mg/kg iv twice weekly; Avastin (Roch) 5 mg/kg iv twice weekly, or IgG control 4 mg/kg iv twice weekly. Tumors were harvested when the tumor diameter reached 2 cm or at 28 days. Tumor volumes were measured with a caliper. Growth rates (percent change per day) were compared with the formula \( (\text{Final volume} - \text{initial volume}) / \text{initial volume} \times 100 \) / number of days. Differences in growth rate between treated and control were analyzed with a two-tailed t-test.

**Results:** PK and PD: SFRP2 MAb was long circulating in the blood with an average \( t_{1/2} \) in the range of 53-89 hr. In addition, the SFRP2 MAB was found to preferentially target the tumors versus all other organs except for the liver. For example, in tumor bearing mice, the blood/tissue ratio on day 14 was smallest in the liver (16:1) and tumor (39:1 to 255:1) proving that the tumor was a prime organ for accumulation of the SFRP2 MAb. SFRP2 MAb was found to preferentially target the tumors versus all other organs including the liver. For example, in tumor bearing mice, the blood/tissue ratio on day 14 was smallest in the liver (16:1) and tumor (39:1 to 255:1) proving that the tumor was a prime organ for accumulation of the SFRP2 MAb. In tumor bearing and non-tumor bearing mice exhibited dose-independent kinetics as a one-way ANOVA analysis comparing \( t_{1/2} \) at different dose levels was not statistically significant (p=0.2847 and 0.1204, respectively). However, there was statistically significant difference in \( t_{1/2} \) of the SFRP2 MAb in tumor-bearing and non-tumor-bearing mice (p=0.0386). **Efficacy in triple negative breast cancer:** There was a 40% decrease in growth rate between SFRP2 MAb and control (p=0.03) and a 20% inhibition of growth rate between Avastin and control (p=0.40). The IgG negative control had no effect on tumor growth.

**Conclusion:** The SFRP2 MAb was long circulating and the tumor was a prime organ for accumulation of the SFRP2 MAb. SFRP2 MAb slowed the growth of a human triple negative breast cancer xenograft in a tumor model that was not sensitive to Avastin. We conclude that SFRP2 is a novel therapeutic target for breast cancer.

**P3-16-05**  
**A Phase II Trial Expansion Cohort of the PARP Inhibitor Veliparib (ABT888) and Temozolomide in BRCA1/2 Associated Metastatic Breast Cancer.**

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**Background:** Veliparib (ABT-888) is a novel oral inhibitor of Poly (ADP-Ribose) Polymerase (PARP) 1 and 2. Veliparib and temozolomide (TMZ) are synergistic in breast cancer xenograft models. We recently conducted a phase II study of TMZ and veliparib in 41 patients (pts) with metastatic breast cancer (MBC). Activity was observed only in pts with known BRCA1 or BRCA2 deleterious mutations, with a response rate (RR) of 50% (4/8) and clinical benefit rate (CBR) of 62.5%. In order to further evaluate the activity and safety of TMZ and veliparib, we enrolled an expansion cohort of 20 additional patients with BRCA1 or 2 mutations.

**Methods:** We previously conducted a single arm phase II trial of veliparib and TMZ in 41 MBC pts. Eligibility included measurable disease; ≥1 prior MBC therapy and PS ≤ 2. Available archived tumor samples were collected. In this expansion cohort, first line therapy for MBC and prior PARP inhibitor therapy were allowed, and eligible patients were required to have a known deleterious BRCA1 or BRCA2 mutation identified from prior clinical testing. The dose of veliparib in the original cohort was reduced from 40 mg PO BID to 30 mg PO BID. In the expansion cohort all patients received veliparib (30 mg PO BID days 1-7) and TMZ (150mg/m² PO QD days 1-5) on a 28 day cycle. RECIST response was evaluated every 2 cycles. The primary endpoint was overall response rate. Secondary endpoints included PFS, OS, safety and toxicity.

**Results:** Between June 24, 2010 and Sept 29, 2010, 20 eligible pts (median age 42) were enrolled. Baseline characteristics included: median PS=0 (range 0-2); 9 BRCA1 carriers, 9 BRCA2 carriers, and 2 unknown. 17 pts (85%) received prior adjuvant chemotherapy. 9 patients received a prior platinum chemotherapy. The most common grade 3/4 toxicities included thrombocytope尼亚 and neutropenia. Best response for the 20 patients evaluable at the time of abstract submission includes 3 PR (15%), 6 SD (30%), 11 PD, and a clinical benefit rate of 45%. Combined with the initial cohort of 8 known carriers from the original 41 patients, the total RR is 25% (7/28) and clinical benefit rate of 50% (7 PR, 7 SD). The RR was 40% (6/15) in pts without prior platinum treatment, and 9% (1/11) in pts with prior platinum treatment. The median PFS for the 20 BRCA carriers was 85 days, and among patients with prior platinum treatment compared to no prior platinum, the median PFS was 70 and 179 days, respectively. Three pts remain on study, 1 with a CR for >20 mo.

**Discussion:** We previously demonstrated that veliparib and TMZ is an active combination in BRCA1/2 associated MBC. In this larger expansion cohort of 20 additional patients, the combination continued to show activity, although the response rate was not as robust as previously observed. Differences between the original cohort and the expansion cohort may account for some variation in response, such as prior platinum or PARP inhibitors therapy, number of lines of prior therapy, and the dose of veliparib used (40mg vs 30 mg). These results
support further evaluation of this regimen in the BRCA1/2 carrier population, and provide the opportunity to evaluate potential factors that may predict response or resistance to this regimen.

**P3-16-06**

**Phase II Trial of TS-1 in Combination with Oxaliplatin (SOX) in Patients with Metastatic Breast Cancer (MBC) Previously Treated with Anthracycline and Taxane Chemotherapy [TORCH]**

[Korean Cancer Study Group (KCSG) BR07-03].

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**Background:** Oxaliplatin, a platinum analogue, is an active drug in advanced anthracycline and taxane-pretreated breast cancer patients as a single agent and with 5-fluorouracil (5-FU) combination. TS-1 was developed by the scientific theory of both potentiating antitumor activity of 5-FU and reducing gastrointestinal toxicity. This trial was performed to evaluate the efficacy and safety of TS-1 in combination with oxaliplatin in metastatic breast cancer (MBC) patients previously treated with anthracycline and taxane chemotherapy.

**Methods:** Between October 2007 and October 2009, MBC patients were enrolled in this prospective multicenter trial. Eligible criteria included age ≥18 years, at least one measurable lesion, prior treatment with anthracycline and taxane chemotherapy, and ECOG Performance Status 0–2. TS-1 40 mg/m² b.i.d. on days 1–14 with oxaliplatin 130 mg/m² on day 1 were administered every 3 weeks till disease progression. Primary end-point was response rate, and secondary end-points were time-to-progression (TTP), overall survival (OS), duration of response (DOR) and toxicities. Response was evaluated every 6 weeks according to the RECIST criteria v.1.0 and toxicity was assessed with NCICTCAE v.3.0. (ClinicalTrials.gov identifier NCT00527930).

**Results:** A total of 87 patients were enrolled. Median age was 48 years (range 30–71 years). Nineteen patients (21.8%) had de novo stage IV and 68 patients (78.2%) had recurrent disease. Thirty-five patients (40.2%) received two-line of prior chemotherapy in palliative setting. Forty-eight patients (55.2%) had ≥3 disease sites. Fifty-five patients (62.1%) were hormone receptor positive, and 25 patients (28.7%) were triple negative. Five patients received prior anti-HER2 therapy. A total of 525 cycles were administered (median 6 cycles, range: 1–22 cycles). In protocol analysis, overall response rate was 38.5% (95% CI: 27.7–49.3) (CR 0%, PR 38.5%) and disease control rate (CR, PR, and SD) was 67.9% (95% CI: 57.5–78.3). Median TTP, OS, and DOR were 6.0 months (95% CI: 5.1–6.9 months), 19.4 months (95% CI: not estimated), and 6.6 months (95% CI: 3.7–9.6 months), respectively. RR was not different by triple negativity (39.1% in TNBC vs. 38.2% in non-TNBC, P=0.361). TTP was not different according to the number of prior chemotherapy regimens. Reported grade 3 or 4 toxicities (per cycle) were neutropenia (10.3%), thrombocytopenia (5.5%), diarrhea (1.9%), vomiting (1.9%), and stomatitis (0.2%). There was no treatment-related death.

**Conclusions:** SOX is an effective regimen in anthracycline and taxane pretreated MBC patients with manageable toxicities.

**P3-16-07**

**Denosumab in Patients with Breast Cancer and Bone Metastases Previously Treated with Zoledronic Acid or Denosumab: Results from the 2-Year Open-Label Extension Treatment Phase of a Pivotal Phase 3 Study.**

Stopeck AT, Lipton A, Martin M, Body J-J, Paterson A, Steger GG, Tonkin K, de Boer RH, Fujiwara Y, Yardley D, Jassem J, Takano T, Solal-Celigny P, Fan M, Braun A. University of Arizona Cancer Center, Tucson, AZ; Penn State Milton S. Hershey Medical Center, Hershey, PA; Hospital General Universitario Gregorio Marañón, Madrid, Spain; CHU Brugmann, Université Libre de Bruxelles, Brussels, Belgium; Tom Baker Cancer Centre, Calgary, AB, Canada; Medical University of Vienna, Vienna, Austria; Cross Cancer Institute, Edmonton, AB, Canada; Western and Royal Hospitals, Melbourne, Australia; National Cancer Center Hospital, Tokyo, Japan; Sarah Cannon Research Institute, Nashville, TN; Tennessee Oncology, PLLC, Nashville, TN; Medical University of Gdansk, Gdansk, Poland; Toranomon Hospital, Tokyo, Japan; Clinique Victor Hugo, Centre Jean Bernard, Le Mans, France; Amsgen, Inc., Thousand Oaks, CA

**Background:** Primary results from a phase 3, randomized, double-blind double-dummy trial showed that compared with zoledronic acid (ZA), denosumab reduced the risk of developing a first on-study SRE by 18% (hazard ratio, 0.82; 95% CI 0.71 to 0.95; P = 0.01), and the risk of multiple SREs by 23% (rate ratio, 0.77; 95% CI 0.66 to 0.89; P = 0.001) in patients with breast cancer and bone metastases. Based on superior efficacy and favorable safety data from the study’s primary analysis (Stopeck et al, 2010), all patients who remained on treatment were offered open-label denosumab in a prespecified 2-year extension treatment phase.

**Materials and Methods:** A total of 2046 patients with breast cancer and bone metastasis were randomized to receive either subcutaneous (SC) denosumab 120 mg and intravenous (IV) placebo or IV ZA 4 mg (adjusted for renal function) and SC placebo Q4W in the double-blinded treatment phase. Patients who completed the double-blinded treatment phase were offered open-label denosumab Q4W for up to 2 years from the start of the open-label treatment phase. Patients who did not participate in the open-label treatment were followed for survival every 12 weeks for up to 2 years after their last dose of investigational product in the double-blinded treatment phase.

**Results:** Of the 752 patients who completed the double-blinded treatment phase, 667 (89%) patients entered the open-label treatment phase: 325 (48.7%) initially randomized to the denosumab group (DD) and 342 (51.3%) to the ZA group in the double-blinded treatment phase (ZD). Demographics were comparable between groups. The total median (Q1, Q3) cumulative denosumab exposure (including extension treatment phase) for DD patients was 19.3 months (9.2, 32.2) (range 0.9–59.8 months). Adverse events (AEs) were comparable between groups (n = 283/318 [89%] for DD patients; n = 303/334 [91%] ZD patients). An additional 20 patients (n = 18 patients in the DD group and 18 patients in the ZD group reported osteonecrosis of the jaw, resulting in a cumulative incidence of 4.7% for DD patients; n = 303/334 [91%] ZD patients). An additional 20 patients (n = 20 patients in the DD group and 18 patients in the ZD group reported osteonecrosis of the jaw, resulting in a cumulative incidence of 4.7% for DD patients; n = 303/334 [91%] ZD patients).

**Conclusions:** Denosumab effectively reduces the risk of SRE by 18% (hazard ratio, 0.82; 95% CI 0.71 to 0.95; P = 0.01) and multiple SREs by 23% (rate ratio, 0.77; 95% CI 0.66 to 0.89; P = 0.001) in patients with breast cancer and bone metastases. Based on superior efficacy and favorable safety data from the study’s primary analysis (Stopeck et al, 2010), all patients who remained on treatment were offered open-label denosumab in a prespecified 2-year extension treatment phase.
Overall survival was similar between groups over the entire study: median 34.4 months (95% CI 31.5 to 39.3) for DD patients, 34.2 months (95% CI 31.0 to 37.6) for ZD patients.

**Conclusion:** A two-year open-label extension treatment phase confirmed the long-term safety profile of denosumab in these breast cancer patients with bone metastases who continued receiving denosumab for up to 5 years or who switched from ZA to denosumab. No new safety signals were observed with up to 5 years of monthly denosumab therapy.

**P3-16-08**

**A Phase 2, Randomized Open-Label Study of Iniparib, Administered Either Weekly or Twice-Weekly in Combination with Gemcitabine Plus Carboplatin in Patients with mTNBC.**

Dièras V, Bonnefoi H, Alba E, Avada A, Coudert B, Pivot X, Gligorov J, Jäger A, Gianni L, Lindeman G, Pham N, Su Y, Gao G, Mery-Mignard D, Paridaens R, Verweij J, Institut Curie, Paris, Cedex 05, France; Université de Bordeaux, Bordeaux, INSERM, France; Hospital Clinico Universitario Virgen de la Victoria, Málaga, Spain; Institut Jules Bordet, Centre des Tumeurs de l’Université Libre de Bruxelles, Brussels, Belgium; Centre Georges François Leclerc, Dijon, France; University Hospital Jean Minjoz, Besançon, France; University Paris VI, Paris, France; Erasmus University Medical Center, Daniel den Hoed Cancer Center, Rotterdam, Netherlands; InSR - San Raffaele, Milan, Italy; The Royal Melbourne Hospital and The Walter and Eliza Hall Institute of Medical Research, Melbourne, Victoria, Australia; Sanofi, Vitry, Paris, France; Sanofi, Great Valley, PA; Sanofi, Cambridge, MA; University Hospital Ghent University, Catholic University of Leuven, Leuven, Belgium; Erasmus University Medical Center; Rotterdam, Netherlands

**Background:** Iniparib (BSI-201) is an investigational anticancer agent whose precise mechanism of action is under active investigation. In breast cancer cell lines and xenograft models of triple-negative breast cancer (TNBC), iniparib exhibits anti-proliferative activity and potentiates the cell cycle effects of some DNA damaging agents. In a randomized, open-label phase 2 study in pts with metastatic TNBC (mTNBC), iniparib combined with gemcitabine (G) and carboplatin (C) (GC) improved efficacy outcomes compared with GC alone. A confirmatory phase 3 study with GCI failed to meet pre-specified criteria for PFS and OS; however, an exploratory subset analysis demonstrated a potential benefit amongst 2/3rd line pts (O’Shaughnessy et al. ASCO 2011). Here we report results of a randomized phase 2 study (NCT01045304) in pts with mTNBC, which assesses efficacy and pharmacokinetics (PK) of iniparib administered either biw or qw in combination with GC.

**Patients and methods:** Eligible pts (N=163; median age 49 yrs) had documented and measurable TNBC, ECOG PS 0-1, normal organ/marrow function, and had received ≤2 prior chemotherapy (CT) regimens for metastatic disease. Pts were randomized (1:1) to receive G (1,000 mg/m², IV, d 1, 8) plus C (AUC 2, IV, d 1, 8) and iniparib either biw (5.6 mg/kg, IV d 1,4,8,11) or qw (11.2 mg/kg, IV d 1,8) on a 21 d cycle. Pts were stratified according to prior CT for mTNBC (0 vs. ≥1). The primary efficacy endpoint was overall response rate (ORR; CR + PR); secondary endpoints included: clinical benefit rate (CBR; CR + PR + SD for 24 weeks), PFS, OS and PK.

**Results:** At the time of analysis, 23% of patients were still on treatment. The median number of cycles administered per patient was 6 in both arms; exposure to iniparib was identical. Safety data are not fully validated. All pts experienced at least 1 treatment emergent adverse event (TEAE). Grade (Gr) ≥3 TEAEs occurred in 94% and 85% of pts in the biw and qw arms, respectively. TEAEs Gr ≥3 occurring in ≥5% of pts regardless of relationship to study drug (biw vs qw) are as follows: blood and lymphatic 71% vs 67%; hepatobiliary 7.5% vs 9.8%; asthenia/fatigue 7.5% vs 11%; GI 8.8% vs 8.5%; infections 7.5% vs 3.7%; respiratory, thoracic and mediastinal 5% vs 8.5%, metabolism and nutrition 4% vs 6%. For response data see table.

**No major difference was observed in drug exposure (based on AUC within one cycle) between the two dosing regimens.**

**Conclusion:** Dosing of GCI on a qw schedule produced a similar ORR to that obtained with the biw schedule. A comparable safety profile in both arms, and consistency with results of previous studies, suggests that the weekly combination of GCI may be an appropriate schedule for further studies evaluating this combination. OS and PFS data are not yet mature; updated efficacy and safety data will be presented.

**P3-16-09**

**Endoxifen, a Newly Developed Breast Cancer Drug, Has Anabolic Actions on the Mouse Skeleton.**


**Background:** Commonly used endocrine therapies for breast cancer, such as aromatase inhibitors in postmenopausal women and tamoxifen in premenopausal women, have deleterious effects on bone mineral density. Therefore, the identification of novel cancer therapies which either maintain or improve bone mass are of clinical need. We have recently demonstrated that endoxifen is the most active tamoxifen metabolite with regard to inhibiting the growth of ERα+ breast cancer cells and these studies have led to the development of endoxifen as a novel anti-breast cancer drug for which first-in-human studies are now underway. At present, there are no data regarding endoxifen’s effects on bone.

**Methods:** The effects of endoxifen on osteoblast (OB) and osteoclast (OC) maturation and gene expression were monitored by cell differentiation assays and real-time PCR. Dual-energy X-ray absorptiometry (DXA), peripheral Quantitative Computed Tomography (pQCT) and micro-Computed Tomography (μCT) were used to determine changes in bone density, mass and architecture following 45 days of oral endoxifen administration (50mg/kg/day) to 3-month-old ovariectomized (OVX) C57BL/6 mice relative to vehicle control treated animals. Alterations in the numbers and activity of OBs and OCs were determined by histomorphometry and serum levels of P1NP and CTX-1 respectively.

**Results:** Endoxifen treatment of mouse derived bone marrow stromal cells and human OBs led to significant increases in the expression of critical bone marker genes such as Runx2, osterix, osteocalcin, osteoprotegerin and alkaline phosphatase in a dose dependent manner. Daily administration of endoxifen to OVX mice led to significant increases in total body bone mineral density (BMD) (6%) and content (BMC) (9%), which was accompanied by a 50% decrease in fat tissue mass as determined by DXA; pQCT analysis of the tibial metaphysis revealed dramatic increases in BMD (35%) and BMC (20%), as well
as trabecular density (52%), cortical content (62%), cortical area (60%) and cortical thickness (78%). μCT analysis of the femoral metaphysis revealed increases in bone volume/total volume (200%), trabecular number (38%) and trabecular thickness (18%), as well as decreased trabecular spacing (29%). Interestingly, there was nearly a 50% increase in the numbers of OCs derived from endoxifen treated mice which was associated with elevated expression of OC marker genes such as NFATc1, RANK, c-fms and cathepsin-K compared to control treated animals. Approximately 4 times as many OBs and OCs were observed on the bone surfaces of endoxifen treated mice which correlated with nearly 2-fold increases in serum levels of the bone formation (P1NP) and resorption (CTX-1) markers.

**Conclusions:** These data are the first to demonstrate that endoxifen has anabolic effects on the mouse skeleton which are similar to that of estrogen. Additionally, these data reveal that endoxifen’s mechanism of action in bone is different than that reported for tamoxifen and other selective estrogen receptor modulators in mice as it increases, rather than decreases, bone formation and remodeling. Therefore, the use of endoxifen for the treatment of endocrine responsive breast cancer may avoid the detrimental skeletal effects of many conventional endocrine therapies.

**P3-16-10**

**The Efficacy of Zoledronic Acid in Breast Cancer Adjuvant Therapy: A Meta-Analysis of Randomized Controlled Trials.**

Lu J, Yan T, Yin W, Zhou Q, Zhou L, Jiang Y, Du Y, Shao Z. Fudan University Shanghai Cancer Center, Shanghai, China; Shanghai Medical College, Fudan University, Shanghai, China

**Background:** The effect of zoledronic acid in breast cancer adjuvant therapy concerning improvement of patient survival has yet to be confirmed. We performed a meta-analysis of published and unpublished randomized controlled trials with the aim of accurate evaluation between clinical outcome and the association of the addition of zoledronic acid to adjuvant therapy.

**Methods:** We searched Pubmed (from 1966 to present) and online abstracts from the proceeding Annual Meetings of the American Society of Clinical Oncology (ASCO) (years 1992-2010) and online abstracts from San Antonio Breast Cancer Symposium (years 2004-2010). A total of five eligible studies including 3676 subjects and 3678 controls met our search criteria and were evaluated. Random and fixed-effects meta-analytical models were used where indicated, and between-study heterogeneity was assessed. The primary study endpoints were the DFS. Secondary endpoints were OS, distant or loco-regional recurrence free survival and bone metastasis free survival.

**Results:** Compared with the control arm, adjuvant breast cancer treatment with zoledronic acid did not significantly improve overall survival (OS), disease free survival (DFS), bone metastasis free survival, distant and locoregional recurrence free survival. However, in the postmenopausal subgroup, the addition of zoledronic acid to standard therapy could significantly improve DFS (RR=0.763, 95%CI 0.658-0.884, p<0.001) and reduce the risk of distant (RR=0.744, 95% CI 0.611-0.906, p=0.003) and locoregional recurrence (RR=0.508, 95% CI 0.340-0.760, p=0.001).

**Discussion:** Adjuvant zoledronic acid may potentially improve the prognosis of postmenopausal patients. Additional studies are needed to evaluate the value of adjuvant treatment of zoledronic acid in premenopausal counterparts, differing disease stages, and various pathological types of breast cancer.

**P3-16-11**

**Prospective Evaluation of Radiation Pneumonitis in Neoadjuvant Concurrent Docetaxel and Radiation Therapy for Locally Advanced Breast Cancer.**


**Background:** Taxanes are known to have radiosensitizing properties, by causing cell arrest in the G2 and M phases of the cell cycle. As taxanes have become integrated into routine oncologic use, concerns have arisen over the association between taxanes and radiation toxicities such as pneumonitis. Pneumonitis has been reported to occur both with taxane administration alone or, more commonly, with concurrent or sequential radiation. The purpose of this study is to evaluate radiation pneumonitis from our institutional phase I/II protocol of neoadjuvant FEC chemotherapy followed by weekly docetaxel concurrent with radiotherapy in the treatment of locally advanced breast cancer (LABC).

**Materials and Methods:** Since August 2009, thirty-two LABC patients with stage IIB, IIIA, IIIB, or IIC invasive breast cancer were enrolled to receive protocol based treatment consisting of 3 cycles of intravenous (IV) fluorouracil 500 mg/m², epirubicin 100 mg/m², and cyclophosphamide 500 mg/m² (FEC) every 3 weeks. Following this, weekly IV docetaxel 35 mg/m² was administered concurrently with locoregional external beam radiotherapy to a total dose of 45 Gy in 25 fractions followed by a boost of 5.4-9 Gy in 3-5 fractions to gross residual disease. Adverse events were scored using the National Cancer Institute Common Terminology Criteria for Adverse Events v3.0. A linear regression model was built used to evaluate potential parameters predictive of clinical pneumonitis (grade ≥2).

**Results:** Of the 32 patients enrolled on this prospective protocol, 7 were excluded from analysis (n = 6, follow-up < 4 weeks; n = 1, converted to palliative radiotherapy due to metastatic disease). Twenty-five patients remained for analysis. The median age was 48 years (range 26 to 64). Thirteen patients were treated with intensity modulated radiation therapy while 12 were treated using 3D conformal radiotherapy. In total 13 patients (52%) experienced clinical pneumonitis with 6 of these patients (24%) transiently requiring supportive oxygen (grade 3). On linear regression modeling, the use of IMRT (p = 0.08), grade 3 skin toxicity (p = 0.08), and baseline left ventricular ejection fraction (LVEF, p = 0.05) were potentially predictive of symptomatic pneumonitis. Conversely, various heart and lung dose-volume histogram parameters, trastuzumab use, bolus use, disease laterality, total docetaxel dose, were not predictive of symptomatic pneumonitis (p > 0.10). In multivariable modeling, the use of IMRT (p = 0.05) and baseline LVEF (0 = 0.03) remained predictive of symptomatic pneumonitis.

**Conclusion:** The use of concurrent weekly docetaxel-based chemoradiotherapy in LABC is associated with significant symptomatic pneumonitis and may be related to low baseline LVEF and the use of IMRT. However, conventional parameters of low dose volumes of lung and heart irradiated were not predictive of pneumonitis. The relationship of the use of IMRT and taxanes in the development of pneumonitis is likely complex and warrants further investigation.

**P3-16-12**

**Abstract has moved to PD08-11**
P3-16-13
Cardiac Safety of Non-Pegylated Liposomal Doxorubicin and Docetaxel as 1st Line Treatment in Metastatic HER2 Negative Breast Cancer (Myotax Study).

van Warmerdam L, Schiphorst P, Nieboer P, Derksen M, de Jongh F, Schmidt R, Catharina-Ziekenhuis, Eindhoven, Netherlands; Streekziekenhuis Koningin Beatrix, Winterswijk, Netherlands; Wilhelmina Ziekenhuis, Assen, Netherlands; Maxima MC, Eindhoven, Netherlands; IkaZie Ziekenhuis, Rotterdam, Netherlands; Foundation BO3, Steenderen, Netherlands

Background: Doxorubicin (DOX) is an effective, but cardiotoxic agent in metastatic breast cancer (BC). Incidence of heart failure (HF) is 2-4% and increases considerably with cumulative doses over 450-550 mg/m². In Myocet® DOX is encapsulated in liposomes. It is delivered predominantly to areas with increased capillary permeability such as tumors and reduces cardiac exposure. We conducted an open non-comparative study to assess cardiac safety of Myocet® combined with docetaxel.

Materials and methods: Females with locally advanced or metastatic HER2 negative BC. 6 cycles of Myocet® 60 mg/m² and docetaxel 75 mg/m² q3w as 1st line therapy. Left ventricular ejection fraction (LVEF) and disease status were assessed after cycle 2, 4, and 6. Primary endpoint: signs and symptoms of HF (NYHA III-IV) or LVEF <50% and decrease ≥5% (with symptoms) or ≥10% (without symptoms).

Results: 68 patients (pats) were included. Mean (sd;range) age 56.3 y (10.2;32-79), mean disease duration 5.5 y (5.1 y;1 mo-19 y), 31 pats had anthracyclines (AN) in the past. 49 pats completed all 6 cycles. Mean LVEF (%) over time are presented in Table 1.

<table>
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<tr>
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<th>After cycle 2</th>
<th>After cycle 4 6</th>
<th>After cycle 4 8</th>
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<tr>
<td>Baseline</td>
<td>63.4 (17.25)</td>
<td>63.9 (6.825)</td>
<td>63.0 (7.231)</td>
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<tr>
<td>AN pretreated</td>
<td>79.2 (17.25)</td>
<td>81.6 (7.231)</td>
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</tr>
<tr>
<td>Not AN pretreated</td>
<td>63.4 (17.25)</td>
<td>63.9 (6.825)</td>
<td>63.0 (7.231)</td>
</tr>
</tbody>
</table>

Conclusion: Liposomal DOX might provide more cardiac safety than conventional AN and a DNA damage response in HCC1187 (representative of the most resistant group), and induced a strong effect of apoptosis and cell cycle, apoptosis and DNA damage responses were analyzed by western blotting. Studies on the action of TG02 in combination with chemotherapy were performed in MDAMB231 and HBL100 cells, and the potency of the combination was quantitated with the Calcusyn software. In vivo studies on the action of TG02 were performed in mice xenografted with the TNBC cell line MDA-MB231.

Results
The TNBC cell lines analyzed showed high levels of Erk5 expression, and Erk5 was active under resting conditions in some of them. TG02 inhibited the kinase activity of Erk5 even though TG02 did not affect the Erk5 upstream activating kinase Mek5. TG02 showed an inhibitory effect of phosphorylation of residue Thr732 in the C-terminal tail of Erk5 without affecting the phosphorylation of the activation loop TEY motif. Cell proliferation studies indicated that one group of TNBC cells were very sensitive to the action of this compound (IC50 ≤100 nM) and another group were more resistant. TG02 induced cell cycle arrest at the early G1 and G2 phases of cell cycle, and triggered cell death in MDAMB231 (representative cell line of the most resistant group), and induced a strong effect of apoptosis and a DNA damage response in HCC1187 (representative of the most sensitive group). In vitro studies indicated that TG02 sensitizes TNBC cells to chemotherapy, showing additive or synergistic effects depending of the doses. In vivo studies indicated that TG02 exerted a strong antitumoral action in mice bearing MDA-MB231-derived tumours.

Conclusions
TNBC cells are very sensitive to TG02, both in vitro and in vivo. The inhibits the kinase activity of Erk5, which, together with the targeting of other kinases, may contribute to the induction of cell cycle arrest or apoptosis in response to the compound in TNBC cells. TG02 synergized with chemotherapy, supporting the possibility of using this drug in combination therapy. Taken together, these preclinical studies establish the bases for the clinical development of this compound for the treatment of TNBC.

P3-16-14
Effect of TG02, a Multikinase Inhibitor, on Triple Negative Breast Cancer Cells.

Pandiella A, Ortiz-Ruiz MJ, Burrows F, Ocaña A, Esparis-Ogando A. Cancer Research Center, Salamanca, Spain; Hospital Universitario, Albacete, Spain; Tragara Pharmaceuticals, San Diego, CA

Background
Breast cancer is the most common neoplasm in women. Formerly, we reported that the MAPK Erk5 participates in the proliferation of breast cancer cells in vitro, it is overexpressed in the tumours of a number of breast cancer patients, and its overexpression is an independent prognostic marker for disease-free survival. In addition, inhibition of Erk5 sensitized cells to treatments commonly used in the breast cancer clinic. Therefore, Erk5 may represent a novel therapeutic target in breast cancer. Here we describe the preclinical activity of TG02, a novel multi-kinase inhibitor being developed by Tragara Pharmaceuticals, in triple negative breast cancer (TNBC). TG02 presents a unique kinase inhibitory spectrum, combining Erk5 inhibitory properties with inhibition of CDKs and certain receptor tyrosine kinases.

Material and methods
The action of TG02 on cell proliferation of TNBC cell lines was carried out by the MITT-based assay, and its action on cell death and cell cycle progression was analyzed by flow cytometry. The expression of different kinases and other proteins implicated in cell cycle, apoptosis and DNA damage responses were analyzed by western blotting. Studies on the action of TG02 in combination with chemotherapy were performed in MDAMB231 and HBL100 cells, and the potency of the combination was quantitated with the Calcusyn software. In vivo studies on the action of TG02 were performed in mice xenografted with the TNBC cell line MDA-MB231.
Phase I Study with Biomarker Evaluation of Neoadjuvant Sunitinib in Combination with Exemestane in Post-Menopausal Women with Hormone-Sensitive, Her-2 Negative Primary Breast Cancer.

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Background: In preclinical models, oestrogen causes a rapid induction of VEGF in mammary tumours leading to tumour angiogenesis, tumor growth and cell migration whereas aromatase inhibitors (AI) have the opposite effect. Exemestane (E) is an AI, effective in metastatic hormone-dependent breast cancer, as well as in the adjuvant and neoadjuvant settings. Sunitinib (S) is an oral multitargeted tyrosine kinase inhibitor with antiangiogenic activities via inhibition of VEGFR, PDGFR, KIT, RET, CSF-1R and FLT-3, and direct antitumor activity shown in heavily pretreated subjects with advanced breast cancer. The use of antiangiogenic agents at initial stages of the disease, when fewer proangiogenic factors are present, may result in significantly greater efficacy than using these agents at a later stage. Safety profiles of both agents indicate only modest toxicity and do not overlap. We hypothesize that since antiangiogenic and hormonal agents use different mechanisms of action, combination will have additive activity. Regarding (S), continuous dosing (CD) at low doses might result in improved efficacy while maintaining good tolerability.

Methods: This is a phase I study to evaluate the safety of (S) in combination with (E) administered for 24 weeks (1 cycle=4 weeks) as neoadjuvant therapy for post-menopausal women with newly diagnosed hormone-sensitive, Her-2 negative primary breast cancer. We report the results of the first dose escalation part of the study intended to determine a safe dose level of (S) that can be combined with (E) at conventional fixed dose of 25 mg/d. Preliminary efficacy data in terms of objective clinical response by WHO criteria will also be reported. Results: 18 postmenopausal women with ER-positive invasive breast cancer, adequate organ function and ECOG 0-1 were enrolled in the study (median age 74, range 57-84) to be treated at two dose levels: 25 mg of (E) with either 25 mg or 37.5 mg of (S) CD. Two DLTs were identified: grade 3 mucositis at level 25 mg of (S) and grade 3 asthenia at level 37.5 mg of (S). Main toxicities were asthenia, leucopenia, mucositis, diarrhoea and HBP. 78% of patients had grade 2 toxicities; 22% had grade 3. There were no serious adverse events reported. Median time on treatment with the combination was 23 weeks (range 2-24). 16% of patients needed one dose reduction of (S), 16% needed two or more and 28% suspended (S) administration due to toxicity. 50% of patients completed 24 weeks of treatment. None of the patients at 37.5 mg (S) level could tolerate full doses for the entire treatment period, therefore in our study the recommended dosage of (S) that can be safely combined with (E) is 25 mg. Concerning efficacy, 11 out of 18 patients had partial response (61%), 6 out of 18 had SD (33%) and only one patient had PD (5%) as best response. Conclusion: Safety profile of the combination seems to be manageable in most patients. Toxicities were observed mainly within the two first cycles. Response rates were similar than those previously observed with (E) as monotherapy in the neoadjuvant setting. Biomarkers information will be provided.

This study was supported by an Independent Investigator Research grant from Pfizer, Inc.

Overcoming EGFR Resistance Using Dasatinib in Combination with Cetuximab and Cisplatin in Triple Negative Breast Cancer Cell Lines.

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Background: Patients presenting with triple negative breast cancers (TNBC’s) have a poorer prognosis compared to those with other subtypes of breast cancer, and effective therapeutic targets have yet to be identified. The majority of TNBC’s overexpress EGFR, however studies have demonstrated that EGFR inhibition as a monotherapy is ineffective. Interestingly, adding the EGFR inhibitor, cetuximab to cisplatin chemotherapy doubled both the response rate and time to progression compared with patients only on cisplatin. c-Src tyrosine kinase plays a critical role in signal transduction downstream of growth factor receptors, and has been found to contribute to the resistance of TNBC cell lines through the phosphorylation of EGFR. We hypothesize that dasatinib, a c-Src inhibitor, may help overcome EGFR resistance and in combination therapy enhance the cytotoxic activity.

Material and Methods: A panel of breast cancer cell lines, including 7 triple negative cell lines, were tested using increasing doses of dasatinib (1 nM to 10 µM), cetuximab (1 µg/ml to 100 µg/ml), and cisplatin (10 nM to 10 µM) alone and in combination. Growth inhibition was determined after 3 days of treatment using the MTT colorimetric assay, and apoptosis was assessed through Caspase-3 activation and TUNEL assays.

Results: All cell lines were generally resistant to cetuximab, with 100 µg/ml required to decrease cell viability. Two TNBC lines, SUM149 and SUM229, showed an additional decrease in cell viability with dasatinib added to cetuximab, while SUM102 and BT20 cells showed an additional decrease in viability using the dasatinib and cisplatin combination, compared to single agents. In 4 out of 7 TNBC lines, the combination of dasatinib, cisplatin, and cetuximab showed a significant decrease in cell viability compared to the combination without dasatinib (p-value < 0.02). The decrease in proliferation using the combination of dasatinib, cisplatin, and cetuximab was due to an increase in apoptosis. In contrast, this combination did not increase the loss of cell viability or apoptosis in ER/PR+ and HER2+ breast cancer cell lines.

Discussion: Targeting c-Src family kinases with dasatinib may help overcome the resistance to EGFR inhibition in triple-negative breast cancer. This may have significant clinical implications in treating TNBC patients, and hence further investigation into the mechanism of this effect is warranted.

The Identification of Novel Microtubule Stabilizing Taccalonolides.

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Microtubule stabilizing agents, including the taxanes and epothilones, are some of the most important drugs used in the treatment of cancer. All of the microtubule stabilizers used clinically bind within the taxane site on microtubules and work through a similar mechanism of action. We have identified a new class of microtubule stabilizers, the taccalonolides, from plants of the genus Tacca. The most abundant taccalonolides, A and E, are classified as microtubule stabilizers due to their ability to disrupt both interphase and mitotic cellular microtubules, leading to mitotic arrest and apoptosis. However, they are unique from all other microtubule stabilizers in that they do
not bind directly to microtubules or stimulate the polymerization of tubulin in biochemical preparations. The taccalonolide structure is characterized by a highly acetylated pentacyclic steroid skeleton, which is also structurally distinct from all other microtubule-targeted agents. The unique structure and mechanism of action of the taccalonolides, together with their ability to circumvent clinically relevant forms of taxane resistance, prompted our efforts to explore the structure-activity relationship of this class of molecules. We have isolated several novel taccalonolides, designated Z, AA, AB, AC, AF and AG, as well as multiple known taccalonolides. Each of these taccalonolides exhibited cellular microtubule stabilizing activity and antiproliferative actions against breast cancer cells. However, profound differences in potencies were noted with IC50 values ranging from 32 nM to 13 μM. These studies demonstrate that microtubule stabilizing activity is a shared trait among this class of molecules and that significant structure-activity relationships exist. Additionally, in vivo antitumor evaluations of taccalonolides A, E and N demonstrate that each of these molecules has activity in vivo and that taccalonolide A is the most effective against a syngeneic mammary model of breast cancer.

**P3-16-18**

**Phase 2, Open-Label Study of EZN-2208 (PEG-SN38) in Patients with Previously Treated Metastatic Breast Cancer.**

O’Shaughnessy JA, Osborne CRC, Steinberg MA, Holmes FA, Kim HS, Kocs DM, Richards PD, Volkel SJ, Berkowitz N, Buchbinder A. Texas Oncology - Baylor Charles A. Sammons Cancer Center, Dallas, TX; Virginia Oncology Associates, Norfolk, VA; Texas Oncology - Houston Memorial City, Houston, TX; Rocky Mountain Cancer Centers, Denver, CO; Texas Oncology - Austin Midtown, Austin, TX; Oncology & Hematology Associates of Southwest Virginia, Inc., D.B.A. Blue Ridge Cancer Care, Salem, VA; Tyler Cancer Center, Tyler, TX; US Oncology; Enzon Pharmaceuticals, Inc., Piscataway, NJ

**Background:** EZN-2208 is a water-soluble PEGylated conjugate of SN38 that results in parenteral delivery, increased solubility, higher exposure, and longer apparent half-life of SN38, as well as more profound deoxyribonucleic acid (DNA) damage and inhibition of angiogenesis. EZN-2208 results in prolonged exposure of tumors to SN38 via preferential accumulation of EZN-2208 in the tumor and prolonged release of SN38 in the blood.

**Methods:** This trial evaluated EZN-2208 delivered as a 1-h IV infusion weekly for 3 wks in 4-wk cycles. The primary objective was to determine the overall response rate (RR) in female patients with metastatic breast cancer (MBC) who had received prior adjuvant or metastatic therapy with either 1) anthracycline and taxane (AT) or 2) anthracycline, taxane, and capecitabine (Xeloda®) (ATX). Secondary objectives included evaluation of RR based on tumor receptor status, duration of response, progression-free survival (PFS), overall survival (OS), and safety and toxicity. Response was evaluated using RECIST (v1.1).

**Results:** 148 patients received EZN-2208 in the AT (n=65; median age = 56 y [31-84 y]) or ATX (n=83; median age = 55 y [36-83 y]) cohorts. All 65 patients in the AT cohort had received 0-2 lines of prior cytotoxic therapy for MBC; for the ATX cohort, 31 patients (37%) had received 0-2 prior lines of cytotoxic therapy for MBC, 50 patients (60%) had received 3-4 prior lines, and 2 patients (2%) had received 5 prior lines. Preliminary results follow; final data will be presented at the meeting. Median (range) cycles of EZN-2208 was 2.3 (0.3-14) for AT and 2 (0.3-15) for ATX. Best overall response is shown in the table. RR (PR+uPR) was 22% for AT and 10% for ATX. Median (95% CI) time to progression was 3.8 mo (3.6-7.4) for AT and 3.5 mo (1.8-3.7) for ATX. Median (95% CI) duration of response was 4.0 mo (3.7-5.6) for AT and 5.2 mo (1.9-7.3) for ATX. 6-mo PFS (95% CI) was 34% (19%-50%) for AT and 19% (9%-29%) for ATX. Median PFS (95% CI) was 3.8 mo (2.7-5.6) for AT and 2.9 mo (1.8-3.7) for ATX. Median OS (95% CI) was 9.1 mo (6.1-12.7) for AT and 7.9 mo (6.4-12.9) for ATX. Grade 3 or 4 drug-related adverse events (>10% of patients in either arm) included neutropenia (43%, 33%), diarrhea (11%, 8%), and leukopenia (11%, 6%).

<table>
<thead>
<tr>
<th>Best Overall Response, N (%)</th>
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<tbody>
<tr>
<td>AT (N=65)</td>
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<tr>
<td>ATX (N=83)</td>
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<tr>
<td>Response not yet evaluated</td>
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<td>Patients with any receptor status</td>
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<tr>
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<tr>
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<tr>
<td>PR+uPR*</td>
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<tr>
<td>PR+uPR+</td>
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<tr>
<td>CBR**</td>
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<table>
<thead>
<tr>
<th>Patients with triple-negative breast cancer (TNBC)</th>
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<tbody>
<tr>
<td>PR</td>
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<tr>
<td>4 (20)</td>
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<tr>
<td>SD</td>
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<td>11 (55)</td>
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<td>CBR**</td>
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<td>6 (30)</td>
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<tr>
<td>CBR**</td>
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<tr>
<td>3 (23)</td>
</tr>
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</table>

*PR = unconfirmed partial response; **Clinical benefit rate (CBR) = CR+PR or SD +6 months.

**Conclusions:** EZN-2208 is active in patients with previously treated MBC. The activity is similar regardless of ER status and is promising in the TNBC population. The safety profile of EZN-2208 is acceptable with good tolerability in most patients. Further evaluation of EZN-2208 in this population is warranted.

**P3-17-01**

**ApoE and Its Receptors (LRP8, VLDLR) Function as Growth Signals for Triple-Negative Breast Cancer and Represent a Novel Therapeutic Target.**

Shiang C, Qi Y, Wang B, Broom B, Pusztai L. UT M.D. Anderson Cancer Center

Triple-negative breast cancers (TNBC) lack the expression of estrogen, progesterone, and the human epidermal growth factor 2 receptors and have limited treatment options. We hypothesized that genes that are frequently amplified or overexpressed in these cancers are functionally important for the growth and survival of triple-negative breast cancer.

**Materials/Methods:** We identified genes overexpressed in TNBC in 3 different human breast cancer gene expression data sets (n=294, n=286, n=198). We assessed the functional importance of the consistently overexpressed genes using siRNA screen on 18 breast cancer cell lines (10 ER-, 8 ER+) in vitro. We targeted each gene with 4 different siRNA constructs separately and each experiment was performed in triplicate. The genes with the greatest TNBC-specific inhibitory effect after siRNA down regulation were selected for further mechanistic and signal transduction studies.

**Results:** 684 genes showed consistent and highly significant overexpression in TNBC compared to receptor-positive cancers in all 3 data sets. siRNA suppression of 161 of these genes inhibited cell growth significantly more in the ER-negative compared to ER-positive cells by at least 1 of the 4 siRNAs. 27 genes showed similar effect with 2 or more siRNAs and for 2 genes 3 of the 4 siRNAs showed preferential growth inhibition in ER-negative cells. These two genes were VLDLR (very low-density lipoprotein receptor) and LRP8 (low density lipoprotein receptor-related protein 8). We validated the siRNA screen results and confirmed down regulation of the mRNA and protein levels for VLDLR and LRP8 in 4 different ER-negative cell lines and showed that siRNA inhibition can be rescued by co-transfection of the receptor genes. Reanalysis of gene expression
data also indicated expression of the LRPI8 and VLDLR ligands, Reelin or ApoE, in both breast cancer tissues and in cell lines. We next demonstrated that exposure to Reelin and ApoE stimulates the growth of ER-negative cells in vitro. The stimulatory effect of ApoE was isoform dependent ApoE2 (Cys112/Cys112) has the lowest receptor binding affinity and showed no growth stimulation, ApoE3 (Cys112/ Arg158) had modest 50-60% growth stimulation and ApoE4 (Arg112/ Arg158) had the greatest stimulatory effect (300-400%). Suppression of the expression of either LRPI8 or VLDLR or exposure to RAP (an inhibitor of ligand binding to LRPI8 and VLDLR) abolished this ligand-induced proliferation. ApoE4 stimulation results in the transcriptional upregulation of genes involved in proliferation, metabolism, and inflammatory signaling pathways. ApoE4 stimulation increases expression of proteins involved in MAPK/ERK pathway, DNA damage repair, and inflammation. **Conclusions:** We show that the ApoE–LRPI8/VLDLR ligand receptor system is overexpressed in human TNBC. We also demonstrated that this receptor system mediates a strong growth promoting and survival function in TNBC cells in vitro. Interestingly, allelic imbalance favoring ApoE4 expression (E3/E4 or E4/E4) has been linked to higher risk of developing early onset breast cancer, which is primarily TNBC. We propose that inhibitors of LRPI8/VLDLR signaling may be clinically useful therapeutic or preventive agents for TNBC.

**P3-17-02**

**Targeting the Autophagy Pathway for Drug Resistance of Breast Tumor-Initiating Cells.**
Zhao H, Li F, Cui K, Sheng J, Landis M, Chang J, Wong S, Dave B. The Methodist Hospital Research Institute, Weil Medical College, Cornell University, Houston, TX; The Methodist Hospital, Weil Medical College, Cornell University, Houston, TX

**Background:** Our clinical data and experimental evidence reveal that the tumor-initiating cells (TICs) in the original tumor are intrinsically resistant to conventional chemotherapy and radiation therapy and greatly enriched in residual breast cancers after such treatments. We have published a gene expression signature of such breast TICs, and our pathway analysis on the gene signature suggests that the activation of autophagy pathway may be an intrinsic characteristic of the TICs. This motivates us to further investigate the role of the autophagy pathway in TICs self-maintenance and their resistance to hormonal and chemo therapies, as well as their response to TIC-targeted therapies.

**Methods:** A collective 84 well-documented autophagy pathway genes were used to compare the activation of autophagy pathway in different microarray datasets, 1) flow-sorted CD44+/CD24−/low cancer cells vs. all other cells (representing 20 patients), and cancer-derived MSs vs. corresponding primary bulk tumors (representing 17 patients), 2) before vs. after letrozole and doxetaxel treatments (representing 30 patient pairs), 3) before vs. after letrozole treatment (representing 176 patient pairs), and 4) before vs. after lapatinib treatment (representing 115 patient pairs). We applied the Significance Analysis of Microarrays (SAM) algorithm to analyze the expression data of 211 normalized probes for the 84 genes. Levels of TGF alpha were measured by ELISA in conditioned media from cell lines (MCF7, T47D, BT474, JIMT-1, SKBR3, MDA-MB-453). Growth inhibitory effects were determined by cell counts and by MTT cell viability assay (Roche), following 5 days post treatment. Levels of TGF alpha were measured by ELISA in conditioned media from cells treated with 1µM WAY for 24 hours. Levels of pEGFR were also measured by ELISA.

**Results:** We analyzed expression of ADAM17 across molecular subtypes for breast cancer. Levels of ADAM17 mRNA were significantly enriched in the basal like subtype (most of which were TNBC) compared to luminal A, luminal B or HER2 positive breast cancers. In vitro, treatment with WAY-180022 alone resulted in growth inhibitory effects ranging from 15–49% in TNBC cells as determined by cell counts. By MTT viability assay, treatment with 1 µM WAY resulted in a significant reduction in cell proliferation compared to vehicle control in MDA-MB-231 (p = 0.007) cells. Addition of WAY to gefitinib resulted in a significant reduction in proliferation in MDA-MB-231 cells compared to either gefitinib or WAY alone.

**Conclusion:** Activation of the autophagy pathway in TICs is a promising target to combat the drug resistance of breast cancer to conventional systemic therapy.

**P3-17-03**

**ADAM17: A Novel Therapeutic Target for Treatment of Triple Negative Breast Cancer.**
McGowan PM, Mullooly M, Sukor S, Madden S, McDermott E, Pierce A, Crown J, O’Donovan N, Duffy MJ. St. Vincent’s University Hospital, Dublin, Ireland; University College Dublin Dublin, Dublin, Ireland; Dublin City University, Dublin, Ireland; Molecular Therapeutics Cancer Ireland, Dublin, Ireland

**Background:** Due to the absence of ER, PR and HER2, targeted therapy is currently unavailable for patients with triple negative breast cancer (TNBC). Although TNBC lack overexpression of HER2, HER1, or EGFR, signaling is active in at least a subset of these cancers. Activation of EGFR is initiated by ADAM17-mediated release of ligands such as EGF, TGFα and HB-EGF. The aim of this study was to test the hypothesis that inhibition of ADAM17 is a novel approach for the treatment of TNBC.

**Methods:** Gene expression data sets were analyzed for ADAM17 expression (3,519 samples across 21 datasets incorporating 10 different microarrays). We used the ADAM17 specific inhibitor, WAY-180022 (WAY: Pfizer), the EGFR inhibitor gefitinib and 5-fluorouracil (5FU) (Sigma Aldrich) to determine their effects on the growth of 7 TNBC cell lines (MDA-MB-231, MDA-MB-468, SUM159PT, HCC1937, BT20, Hs578T, Hs578T) and 6 non-TNBC cell lines (MCF7, T47D, BT474, JIMT-1, SKBR3, MDA-MB-453). Growth inhibitory effects were determined by cell counts and by MTT cell viability assay (Roche), following 5 days post treatment. Levels of TGFalpha were measured by ELISA in conditioned media from cells treated with 1µM WAY for 24 hours. Levels of pEGFR were also measured by ELISA.

**Results:** We analyzed expression of ADAM17 across molecular subtypes for breast cancer. Levels of ADAM17 mRNA were significantly elevated in the basal like subtype (most of which were TNBC) compared to luminal A, luminal B or HER2 positive breast cancers. In vitro, treatment with WAY-180022 alone resulted in growth inhibitory effects ranging from 15–49% in TNBC cells as determined by cell counts. By MTT viability assay, treatment with 1 µM WAY resulted in a significant reduction in cell proliferation compared to vehicle control in MDA-MB-231 (p = 0.007) cells. Addition of WAY to gefitinib resulted in a significant reduction in proliferation in MDA-MB-231 cells compared to either gefitinib or WAY alone.
(p = 0.017 and p = 0.003, respectively). Similarly, addition of WAY to 5FU resulted in a significant inhibitory effect compared to 5FU alone in MDA-MB-468 (p = 0.007) cells. Investigating the functional effects of ADAM17 inhibition on breast cancer cells, we found that levels of pEGFR were reduced by between 26 – 57.6% in 5 of 6 lines examined. pEGFR levels were significantly reduced in MDA-MB-468 (p = 0.01) and MDA-MB-231 (p = 0.01) cells. In addition, levels of TGFα were significantly reduced following treatment with 1 µM WAY (MDA-MB-468, p = 0.008; MDA-MB-231, p = 0.012; HCC1143, p = 0.033; Hs5788, p = 0.036). Conclusion: Based on our findings, inhibition of ADAM17 with WAY-188022 is a potential treatment for patients with TNBC. Acknowledgement: The authors wish to thank Science Foundation Ireland, Strategic Research Cluster award (08SRC/B1410) to Molecular Therapeutics for Cancer Ireland and the Health Research Board Clinician Scientist Award (CSA/2007/11) for funding this work.

P3-17-04
CXCR1/2 Regulates Human Breast Cancer Stem Cell Activity Via EGFR/HER2-Dependent and -Independent Pathway.
Singh JK, Farnie G, Clarke RB, Hundred NJ. School of Cancer and Enabling Sciences, University of Manchester, Paterson Institute for Cancer Research, Manchester, United Kingdom; University Hospital of South Manchester, Wythenshawe Hospital, Manchester, United Kingdom

Background: Increasing evidence suggests that breast cancers are sustained by breast cancer stem cells (CSCs). Interleukin-8 (IL-8) via its cognate receptors, CXCR1 and CXCR2, and HER2 regulate CSC activity. Cytokine receptors can trans-activate members of the epidermal growth factor receptor (EGFR) family in normal and malignant tissue. The aims of this study were to determine (1) the effect of the IL-8-CXCR1/2 signalling pathway on human breast CSC activity, (2) the effect of CXCR1/2 inhibition in combination with EGFR/HER-2 inhibition on CSC activity and (3) the interaction of CXCR1/2 and EGFR/HER2 in breast cancer.

Methods: Human breast cancer cell lines used were HER2-18 and SKBR3 (both HER2 over-expressing). Primary human breast cancer cells were extracted from metastatic samples (pleural effusion n=4; ascites n=5; local recurrence n=1) and invasive breast cancers (n=1). CSC activity was investigated in vitro using the mammosphere assay. Mammosphere forming efficiency (MFE) was determined by dividing the number of mammospheres >60µm in diameter divided by the number of cells seeded and expressed as a percentage. Data is represented as mean MFE ± standard error of mean normalised by the number of cells seeded and expressed as a percentage. Data is represented as mean MFE ± standard error of mean normalised by the number of cells seeded and expressed as a percentage. Results: Ganetespib displayed potent, low nanomolar activity in vitro. Anticancer activity of ganetespib was further investigated in vivo using breast cancer xenografts.

P3-17-05
Beyond HER2 and Hormonal Agents: The Heat Shock Protein 90 Inhibitor Ganetespib as a Potential New Breast Cancer Therapy.
Friedland JC, Sang J, Modi S, Bradley R, El-Hariry I, Wada Y, Proia DA. Synta Pharmaceuticals, Lexington, MA; Memorial Sloan-Kettering Cancer Center, New York, NY

Background: Ganetespib is a fully synthetic and selective inhibitor of heat shock protein 90 (HSP90), a molecular chaperone recognized as a key facilitator of breast cancer initiation, progression and metastasis. Methods: Preclinical activity of ganetespib across the four major breast cancer subtypes and inflammatory breast cancer was assessed in vitro and in vivo. Modulation of cell proliferation and viability was determined both in monolayer and three-dimensional cultures. HSP90 client protein expression and activity was monitored by Western blot and protein array. To recapitulate clinical dosing, kinetics of client protein destabilization were measured following short exposures to drug in vitro. Anticancer activity of ganetespib was further investigated in vivo using breast cancer xenografts.

Results: Ganetespib displayed potent, low nanomolar activity in luminal (A and B), basal (A and B) and inflammatory breast cancer cell lines grown as monolayers in vitro. BT-474 (HER2 amplified) luminal cells grown as mammospheres in 3D were equally as sensitive to ganetespib as those grown in monolayer. In luminal cells, ganetespib simultaneously disrupted multiple signaling components including the estrogen and progesterone receptor, several receptor and non-receptor tyrosine kinases, as well as the MAPK pathway. Further, ganetespib effectively inhibited AKT, PDK1 and SGK3 activity in PI3KCA mutant cells suggesting that HSP90 is essential for both AKT-dependent and AKT-independent signaling. Clinically relevant exposure times to ganetespib in vitro resulted in potent, long term degradation of HER2. In the basal-like breast cancer cell line MDA-MB-231, enriched in CD44+CD24- stem like cells that commonly display chemotherapeutic resistance and activated JAK2/STAT3 signaling, ganetespib (50 nM) induced significant degradation of JAK2 concordant with loss of both tyrosine and serine phosphorylation of STAT3, followed by cell death. The potent anticancer activity in vitro translated in vivo, where ganetespib was effective in modulating breast cancer xenograft growth as a single agent in both luminal and basal-like breast cancer models. Finally, ganetespib has demonstrated encouraging signs of clinical activity in breast cancer patients, including confirmed partial responses in both a triple negative breast cancer patient and a HER2 positive breast cancer patient.
Conclusions: Ganetespib is a highly potent HSP90 inhibitor that displays preclinical activity in breast cancer due to its ability to simultaneously perturb multiple oncogenic signaling pathways.

**P3-17-06**

Final Results of a Controlled, Randomized 3-Arm Phase II Trial of EndoTAG™-1, a Cationic Liposomal Formulation of Paclitaxel Targeting Tumor Endothelial Cells, in Advanced Triple-Negative Breast Cancer (TNBC).

Awada A, Bondarenko IN, Tarasova O, Bonneterre J, Novara E, Ferrero JM, Bakshi AV, Weidenthaler H, Wilke C, Piccart MJ. Institut Jules Bordet, Brussels, Belgium; Dnepropetrovsk State Medical Academy, Dnepropetrovsk, Ukraine; Academy of Medical Sciences of Ukraine, Kharkov, Ukraine; Centre Oscar Lambret, Lille, France; Instytut im. M. Sklodowskiej-Curie, Gliwice, Poland; Centre Antoine Lacassagne, Nice, France; Kaushalya Medical Foundation, Thane, India; Medigene AG, Martinsried, Germany

**Background**

EndoTAG™-1 (ET) is an innovative therapy of paclitaxel (P) embedded in cationic liposomes displaying antitumor activity by targeting negatively charged activated endothelial cells of tumor vessels.

**Methods**

140 patients (pts) with locally relapsed (r) or metastatic (m) centrally verified TNBC were randomized to i.v. weekly ET (22mg/m$^2$) plus P (70mg/m$^2$) (ET+P), biweekly ET alone (44mg/m$^2$) or weekly P (90mg/m$^2$) in a 2:2:1 ratio. Pts had ≤ 1 prior chemotherapy (CT) for r or m disease and ≥ 6 months (mo) after taxane. A cycle (cy) comprised 3 weeks (w) of therapy and 1 w rest. Pts were treated for a minimum of 4 cy or until disease progression/unacceptable toxicity.

Primary endpoint was progression-free survival (PFS) rate at week 16 based on blinded central imaging and local assessment. Secondary endpoints included median overall survival (mOS), tumor response (RECIST), Quality of Life (QoL) and safety. The study was not powered for intergroup comparisons.

**Results**

Pts baseline characteristics were overall well distributed. Appr. 80% of all pts were treated 1st line for r or m TNBC. Results of PFS rates at week 16 are shown in table. Based on central review, median PFS at week 16 was 4.2 mo for ET+P (95% Cl: 3.5-9.1), 3.4 on ET (2.0-3.8) and 3.7 on P (1.9-6.7). Antitumor activity was highest for ET+P with a clinical benefit rate (complete response (CR) + partial response (PR) + stable disease (SD)) of 52% (26/49 evaluable pts), 31% (15/49) on ET and 36% (9/25) on P. The best overall response (CR, PR, SD at any time) on ET+P was 80% (39/49), on ET 65% (32/49) and 68% (17/25) on P.

Analysis of median OS is shown in table. Analysis of the not predefined subgroup (centrally TNBC + ECOG 0/1 + first line population) showed an impressive median OS of 17.8 mo for ET+P. No differences were reported for QoL. No additional toxicities to those known for ET and P were observed except uncomplicated grade 3/4 neutropenia in the ET+P arm.

<table>
<thead>
<tr>
<th>PFS rate at week 16 / 95% confidence intervals</th>
<th>ET+P</th>
<th>ET</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>mITT, Central review</td>
<td>49.1% (26/54 pts) / 32.3-75.7</td>
<td>34.2% (15/44 pts) / 19.6-51.4</td>
<td>48% (12/25 pts) / 27.5-68.7</td>
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<tr>
<th>ET+P</th>
<th>ET</th>
<th>P</th>
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<tbody>
<tr>
<td>mITT, Local review</td>
<td>47.3% (26/55 pts) / 39.7-64.2</td>
<td>29.8% (17/57 pts) / 18.8-44.6</td>
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<tr>
<th>mOS at cut off date week 41 / 95% confidence intervals</th>
<th>ET+P</th>
<th>ET</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>mITT</td>
<td>55 pts, 13.0 mo / 7.8-23.0</td>
<td>57 pts, 11.9 mo / 7.6-16.3</td>
<td>28 pts, 15.1 mo / 9.7-23.0</td>
</tr>
<tr>
<td>TNBC centrally, EOCG 0/1 and 1st-line treatment</td>
<td>44 pts, 17.8 mo / 10.6-23.0</td>
<td>48 pts, 12.5 mo / 8.4-19.8</td>
<td>24 pts, 10.1 mo / 5.9-23.0</td>
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Conclusions

At cut-off date week 41, the final analysis demonstrated ET+P as a promising combination compared to single agent arms. ET+P was well tolerated. These data will be the basis for a confirmatory phase III study.

Acknowledgments:

B. Glasschoeder, U. Elsasser

**P3-17-07**

EGCG, a Green Tea Antioxidant Suppresses Breast Tumor Angiogenesis and Growth Via Inhibiting the Activation of HIF-1α and NFκB, and VEGF Expression.

Makey KL, Tucker KB, Chinchar E, Miele L, Pei I, Thomas EY, Gu J-W. University of Mississippi Medical Center, Jackson, MS

**Background:** Cancer cells are under greater oxidative stress than normal cells. Limited studies have showed that epigallocatechin-3-gallate (EGCG), a green tea antioxidant can afford protection against a variety of cancer types. The role of EGCG in breast cancer therapy is poorly understood. The present study tests the hypothesis that EGCG as an antioxidant can inhibit the activation of HIF-1α and NFκB, and VEGF expression, thereby suppressing tumor angiogenesis and breast cancer progression.

**Material and Methods:** 16 eight-wk-old female mice (C57BL/6J) were inoculated with 10^6 E0771 (mouse breast cancer) cells in the left fourth mammary gland fat pad. 8 mice received EGCG at 50-100 mg/kg/d in drinking water for 4 weeks. 8 control mice received drinking water only. Tumor size was monitored using dial calipers. At the end of the experiment, blood samples, tumors, heart and limb muscles were collected for measuring VEGF expression using ELISA and capillary density (CD) using CD31 immunohistochemistry. Cultured E0771 cells were used for determining the direct effects of EGCG on proliferation (3H-thymidine incorporation), migration (Matrigel assay), VEGF expression (ELISA), the activation of HIF-1α and NFκB (motif binding assays, Active Motif). MCF-7 and MDA-MB-231 cells were also used for 3H-thymidine incorporation.

**Results:** Oral EGCG treatment significantly reduced tumor weight over the control (0.37±0.15 vs. 1.16±0.30 g; P<0.01), tumor CD (109±20 vs. 156±12 capillary/mm^2; P<0.01), tumor VEGF expression (45.72±1.4 vs. 59.03±3.8 pg/mg; P<0.01), respectively. EGCG treatment reduced plasma VEGF levels over the control mice (26.48±3.76 vs. 40.79±3.5 pg/ml; P<0.01). However, there were no differences in the body weight and heart weight between EGCG and the control groups. EGCG did not inhibit angiogenesis and VEGF expression in the heart and skeletal muscle of mice, compared to the control. EGCG at 50 mol/L significantly inhibited the activation of HIF-1α (0.11±0.02 vs. 0.24±0.02; P<0.01) and NFκB (1.15±0.21 vs. 1.61±0.32; P<0.01) as well as VEGF expression (1752±49 vs. 2254±91 pg/mg; P<0.01) in cultured E0771 cells, compared to the control, respectively. EGCG caused a dose-related inhibition on the proliferation and migration in cultured E0771 cells. EGCG also caused a dose-related inhibition on the proliferation in cultured MCF-7 and MDA-MB-231 cells.

**Discussion:** These findings support the hypothesis that EGCG, a green tea antioxidant, directly targets both tumor cells and tumor vasculature, thereby inhibiting tumor growth, proliferation, migration, and angiogenesis of breast cancer, which is mediated by the inhibition of HIF-1α and NFκB activation as well as VEGF expression. Interestingly, oral EGCG treatment has no effects on the body weight, heart weight, angiogenesis and VEGF expression in breast cancer progression.
the heart and skeletal muscle of mice. This work will have important implications for translating EGCG therapy to human breast cancer treatment and prevention.

P3-17-08
Macroautophagy Protects Breast Cancer MCF-7 Cells from TAM-Induced Apoptosis Via Mitogen-Activated Protein Kinase (MAPK) Pathway.

Hou YF, Ma XY, Liu ZB, Yu SJ, Shao ZM. Cancer Institute, Shanghai, China

Tamoxifen (TAM) has been used ubiquitously for endocrine therapy for the hormone-sensitive breast cancer. Several studies have revealed that tamoxifen treatment induced apoptosis and at the same time tamoxifen increased autophagic levels in MCF-7 cells. The previous studies attempt to elaborate the significance and mechanism of autophagy induced by TAM in breast cancer cells, however, there are contradictions among their conclusions, it is still not clear that autophagy protects MCF-7 from apoptosis or promotes apoptosis. Better understanding of the effect of autophagy induced by TAM in breast cancer cells on apoptosis will be of the important clinical significance in endocrine therapy for breast cancer.

The present study shows that tamoxifen treatment significantly increased autophagic levels by inducing autophagic vacuoles formation in MCF-7 cells observed by means of transmission electron microscopy and enhancing the expression of autophagy marker, microtubule-associated protein light chain 3 measured by Western blot. Our research shows tamoxifen enhanced the phosphorylation of MAPKs when inducing autophagy and autophagy decreased significantly when kinase inhibitors were separately used to inhibit the phosphorylation of ERK, JNK, p38 MAPK. MAPK signal transduction pathway was involved in the process of autophagy in MCF-7 cells.

To investigate whether Estrogen receptor-α participated in autophagy caused by tamoxifen, we constructed Estrogen receptor-α gene ESR/1 shRNA expression vector and it could effectively inhibit the expression of ER-α in MCF-7 cells. Our research shows that autophagy was decreased with the downregulation of ER-α, so we conclude that Estrogen receptor-α also involved in autophagy induced by tamoxifen in MCF-7 cells.

To find out the specific role of autophagy in tamoxifen treated breast cancer cell MCF-7, we inhibited autophagy producing after tamoxifen treatment by pretreating the cells with chloroquine and 3-methyladenine, both commonly used as autophagy inhibitors. Another method for autophagy inhibition was Becline-1 siRNA transfection into MCF-7 cells. Than we stained MCF-7 cells with anti-Annexin V FITC and PI and examined apoptotic rate by flow cytometer and we also detected activity of caspase7 in MCF-7 cells.

The results indicate that inhibition of autophagy by the methods mentioned induced higher apoptotic level, therefore, autophagy protected MCF-7 from apoptosis and inhibiting autophagy may be a new strategy to augment the therapeutic effect of tamoxifen treatment. In our study, we induced breast cancer cell MCF-7 resistant to TAM in vitro and we found much higher autophagic level in TAM resistant cells compared with TAM not resistant MCF-7 cells. We draw the conclusion that inhibition of autophagy induced by tamoxifen may be a new therapy for tamoxifen resistant breast cancer.

Key words: tamoxifen, breast cancer, autophagy, MCF-7 cells, MAPKs, estrogen receptor, inhibition, apoptosis

P3-17-09
Neutralizing the Prolactin Receptor with Therapeutic Antibody LFA102: A Novel Approach for the Treatment of Breast Cancer.


The prolactin receptor (PRLR) is a class I cytokine receptor required for the normal development of the mammary gland and is frequently found to be overexpressed in breast tumors. The polypeptide hormone prolactin (PRL) has been demonstrated to induce PRLR signaling through the Jak/Stat, PI3-kinase/AKT and MAPK pathways, leading to cell proliferation and survival. Mammary gland-specific over-expression of PRL in transgenic mice leads to a higher incidence of ER+ and ER- mammary tumors. In addition, the PRLR locus is the site of frequent viral integrations in MMTV-induced mammary tumors. Elevated serum PRL levels in humans have been correlated with an increased risk for breast cancer, especially for ER+ cases, implicating a role for this hormone in the development of human breast tumors. An analysis of more than 3000 breast tumor specimens indicates that PRLR is expressed with high prevalence (60-70% of tumors) across all breast cancer subtypes, with a trend towards higher expression in ER+ tumors. All of these lines of evidence support the hypothesis that targeting the PRL/PRLR axis may be a new approach for addressing unmet medical need in breast cancer. LFA102 is a Human Engineered™ anti-PRLR antibody of the IgG1 isotype that neutralizes the function of PRLR through a non-ligand competitive binding interaction. LFA102 blocks PRL-induced signaling and proliferation in T47D and MCF7 ER+ human breast cancer cells in vitro, and abolishes PRL-induced phosphorylation of Stat5 in T47D xenograft tumors in vivo. An examination of disaggregated primary human breast tumors ex vivo has indicated that PRL frequently induces signaling through Stat5 in the cells and that LFA102 is capable of completely antagonizing this signaling. LFA102 also neutralizes rat PRLR and the antibody potently regresses PRL-dependent Nb2-C11 pre-T cell lymphoma tumors in vivo. Preliminary data suggests that LFA102 is also capable of inhibiting the growth of carcinogen-induced rat mammary tumors. In vitro studies have shown that LFA102 can also mediate antibody-dependent cellular cytotoxicity (ADCC) and inhibit the PRL-dependent release of the pro-angiogenic factor VEGF from breast cancer cells. Thus, there are multiple potential mechanisms through which LFA102 could show anti-tumor activity in vivo. Preclinical toxicological studies of LFA102 indicate that this therapeutic is well tolerated and exhibits a normal pharmacokinetic profile in relevant animal species. The safety and pharmacokinetics of LFA102 in humans are currently being evaluated in a phase I healthy volunteer trial. At the three dose levels explored so far, no infusion reactions or severe adverse events related to the drug have been reported. Preliminary results suggest that LFA102 has an adequate pharmacokinetic profile for further clinical development. An assessment of LFA102 in a population of metastatic breast cancer patients predicted to have the highest probability of benefit is imminent.

P3-17-10
EpCAM as a Target for Chemical Antibodies in Metastatic Breast Cancer.

Shigdar SL, Duan W. Deakin University, Geelong, Victoria, Australia

EpCAM is a 40kDa glycoprotein that functions as an adhesion molecule in benign epithelium. However, it is over-expressed in nearly all carcinomas, including breast carcinomas, and has been
implicated in the pathogenesis and progression of cancer. In primary breast carcinoma, its over-expression correlates with a diminished overall survival in patients with node-positive disease. Indeed, recent evidence has shown that EpCAM is expressed at a higher level in metastases than in matched primary breast carcinoma. This has led to the suggestion that EpCAM over-expression is associated with aggressive behaviour, and that it could be considered a promising therapeutic target for breast cancer. Several immunotherapy trials are ongoing, though there are limitations to their delivery and distribution, suggesting a smaller and more effective EpCAM targeting molecule is required.

Aptamers, also known as chemical antibodies, are short-stranded DNA or RNA molecules and are selected via the systematic evolution of ligands by exponential enrichment (SELEX). They are 10 to 20 times smaller than antibodies and exhibit superb tissue penetration, thereby indicating their suitability in cancer targeting. In addition, aptamers are more stable, non-immunogenic and non-toxic. Following 12 rounds of SELEX using EpCAM protein as a target, we generated several EpCAM specific RNA aptamers. These were investigated using flow cytometry and confocal microscopy for their ability to bind to three breast cancer cell lines, MCF-7, T47D and MDA-MB-231. Our 19-nt aptamer showed specific binding to all three cell lines. In addition, it also showed superior penetration of breast cancer 3-D spheroid tumor models, as compared to a control aptamer and negative cell lines. This aptamer has also shown efficient internalisation via receptor-mediated endocytosis, suggesting it to be a suitable targeting moiety for drug delivery to both primary and metastatic tumors. The usefulness of this aptamer in clinical applications is being actively investigated.

**P3-17-11**
Dovitinib (TKI258), a Dual Inhibitor of FGFR and VEGFR, Induces Tumor Growth Suppression in Xenograft Models of Primary Human Breast Cancer:
Shi MM, Linnartz R, Versace R, Graus Porta D, Kay A, Dugan M, Novartis Oncology, East Hanover, NJ; Novartis Institutes for BioMedical Research, Basel, Switzerland

**Introduction:** The objective of this study was to evaluate the efficacy of dovitinib (TKI258), a small-molecule dual inhibitor of fibroblast growth factor receptor (FGFR) and vascular endothelial growth factor receptor (VEGFR) in mouse models of human breast cancer. Molecular epidemiological studies have implicated that FGFR1 and FGFR2 gene amplification plays a critical role in breast cancer tumorigenesis. FGFR1 gene amplification correlates with gene expression and is associated with poor prognosis in patients with estrogen receptor-positive (ER+) tumors. Dual FGFR/VEGFR inhibitory activities of dovitinib make it an attractive molecule for targeting breast cancer. **Experimental Design:** Dovitinib was evaluated in 3 primary human breast carcinoma xenograft models with different histological and molecular profiles: a triple-negative breast cancer model (HBCx-15), a HER2+ model (HBCx-5), and an ER+ model (HBCx-3). Dovitinib was administered orally at 20- and 40-mg/kg dose levels (n = 8-10/group), once daily for 21 consecutive days. **Results:** At a dose of 40 mg/kg, dovitinib demonstrated strong antitumor efficacy as a single agent, particularly in the ER+ HBCx-3 and the HER2+ BCx-5 tumor models (AT/TO = −26.42% and AT/AC = 5.09%, respectively), whereas the response was less significant in the triple negative breast cancer HBCx-15 model (AT/AC = 31.7%). **Conclusion:** Dovitinib showed significant antitumor effect in human breast cancer xenograft mouse models. This study provides a strong rationale for clinical investigation of dovitinib in patients with advanced breast cancer, particularly in patients with ER+ and HER2+ disease.

**P3-18-01**
cMET Inhibitor and the Inhibition of Growth of Breast Cancer Cells in Bone Marrow Matrix Environment.
Ye L, Mason MD, Bramble P, Jiang WG. Cardiff University School of Medicine, Cardiff, Wales, United Kingdom; Pfizer Limited, Walton Oaks, Surry, United Kingdom

Background: Hepatocyte growth factor (HGF) is a cytokine that has a diverse but potential role in cancer including breast cancer. HGF, which action is mediated by its specific receptor, cMET, stimulates the aggressiveness of cancer cells by increasing the invasiveness and cellular migration. HGF is also a potent angiogenic factor. Small inhibitors to the HGF receptor are currently investigated in clinical trials of various cancers. In solid tumours which have potency of bone metastasis, HGF and particularly the HGF receptor, cMET, have been found to be particularly over-expressed in tumour cells metastatised to bones.

Materials and Methods. A panel of breast cancer cell lines were used. Cell growth was determined using a colorimetric method. Cell adhesion and migration were investigated using a ECIS model. Bone matrix proteins were prepared from fresh bones. A small inhibitor to human cMET, PF02341066 was used in the present study in all the cell models.

Results. Depending on cell types, cancer cells tended to grow at a faster rate in bone marrow microenvironment than under normal conditions (12% higher for MDA MB 231 cells, for example). While PF02341066 have a concentration dependent effect on the growth of breast cancer, this effect became more marked when cells were growing in the bone marrow matrix. Breast cancer cells migrated rapidly in the presence of bone marrow environment, in comparison with controls. This was also inhibited by the cMET inhibitor. The study further showed that cells over-expressing a molecule linked to bone metastasis, namely ALCAM (1,2), responded more vigorously to cMET inhibitor in the matrix adhesion and cellular migration.

Conclusions. The present study has shown that the HGF receptor inhibitor has an inhibitory effect on breast cancer cells. This is particularly so when cells are grown in bone matrix microenvironment. It is concluded therefore that inhibitors to the HGF receptor may have a particular role in the management of bone metastasis in breast cancer.


**P3-18-02**
A Combination of Pathway-Targeted Inhibitor with DNA-Repair Inhibitor: Preclinical Efficacy of Zactima and Olaparib in Triple Negative Subset of Breast Cancer.
Dey N, Wu H, Sun Y, De P, Leyland-Jones B. Emory University, Atlanta, GA

**Introduction:** Pathway-targeted therapy has not been established for the treatment of Triple Negative (TN) subset of breast cancer (BC). Despite emerging data supporting the use of polyADP-ribose polymerase (PARP) inhibitors, complete and durable responses are rare in TNBC and exploration of additional pathway-targeted...
therapies is needed. The high frequency (72-75%) of EGFR expression in TNBCs suggests that loss of BRCA1 may be coupled, either directly or indirectly, with EGFR overexpression in breast cancer (Van der Groep et al., 2006), and a recent report shows that even a partial loss of BRCA1 leads to an overall increase in EGFR expression in mammary epithelial cells (Burga et al., 2011). We hypothesize that the combined inhibition of growth factor driven EGFR pathway (by Zactima, a small molecule kinase inhibitor of EGFR and VEGFR-2) and DNA-repair pathway (by Olaparib, PARG inhibitor) in the presence of DNA-damaging agent (carboplatin) will have anti-proliferative and anti-migratory effects on triple negative breast tumor (TNBT) cells.

Methods: The effects of Zactima, Olaparib (single or in combination) and carboplatin were studied on: (a) the cell survival/proliferation (MTT, SRB, & cell titer-GLO assay), (b) EGF-induced upregulation of downstream proliferation markers, (c) the cellular signals for apoptosis, (d) fibronectin-directed migration (scratch-assay), (e) the vascular mimicry, and (f) the clonogenic survival (3D-ON-TOP assay) in different TNBT cell lines.

Results: Our in vitro data show that, (1) Zactima (10 µM) blocked EGF-induced phosphorylation of AKT (S473 & T308), S6RP, 4EBP1 and ERK as early as 1 hr (after the treatment), (2) the range of EC50's for Zactima (96 hrs) varied from 5 uM -15 uM (HCC70, HCC1937, MDA-MB231, MDA-MB468, BT20), (3) EGFR over-expressing, PTEN-null, and ATM kinase mutated MDA-MB468 cell line exhibited lowest EC50 for Zactima as compared to other TNBT cell lines and was found to be the most sensitive cell line to Zactima (~0.05 uM) when combined with 10 uM fixed dose of Olaparib, (4) a combination of Zactima with Olaparib plus carboplatin altered an adhesion-dependent cell-survival, increased caspase3 activity (expression of cleaved caspase3 and cleaved PARP) time dependently, inhibited vascular mimicry (at 6 and 24 hrs), blocked fibronectin-directed migration (scratch assay, 24 hrs), changed the organization of actin-cytoskeleton, and suppressed clonogenic growth in different TNBT cells lines.

Conclusion: Zactima alone showed anti-proliferative/pro-apoptotic, and anti-migratory effects in TNBT cells. The combination of Zactima with Olaparib plus carboplatin was most effective in blocking the clonogenic growth of TNBT cells. We are currently pursuing studies to, (1) delineate the relationship between the anti-proliferative effects (clonogenic assay) of Zactima (alone or in combination) and the status of the EGFR/ PI3K pathway using PIK3CA-mutated, PTEN-null and EGFR overexpressed TNBT cell lines, and (2) determine the effect of Zactima on angiogenesis using HUVEC cells, the results of which will be presented in the meeting. This work was generously supported by AVON foundation.

P3-18-04 Pathway Guided Selection of Targeted Inhibitors for Breast Cancer Treatment.


Background: The ability to functionally profile a whole spectrum of pathway proteins in tumor may provide valuable information about the likelihood of drug response and potential mechanisms for drug resistance in breast cancer (BCA) patients. Here we report a comprehensive pathway analysis of membrane associated kinases such as HER1, HER2, HER3, cMET, IGF1R, PI3K and downstream signal transduction proteins including Shc, AKT, ERK, MEK, PDK1, PRAS40, p70S6K and eIF4e in breast cancer cell lines and xenografts as in response to inhibitors targeting the Her1/Her2 and PI3K/AKT and MAPK pathways. For comparison we also analyzed NSCLC lines driven by other receptor tyrosine kinases like EGFR and MET.

Methods: Lysates prepared from KPL4 cells and MCF7 cells both harboring PIK3CA mutations (with HER2-amplification and low level HER2 expression, respectively) treated with 6 inhibitors (an allosteric AKT inhibitor, Lapatinib, Regorafenib, MET inhibitor, an allosteric MEK inhibitor or PI3K inhibitor) targeting PI3K/AKT and MAPK pathways were analyzed for modulations of pathway signal transduction proteins including Shc, AKT, ERK, MEK, PDK1, PRAS40, p70S6K and eIF4e in breast cancer cell lines and xenografts.

Results: Treatment of ER+ and HER2+ cell lines with INK128 in vitro showed that 1) cell lines were sensitive to single agent INK128 with 50% inhibitory concentration ranging from 15 nM-10 µM in ER+ cell lines and 20 nM -50 nM in HER2+ cell lines, 2) 70-80% anti-proliferative activities were observed on 3D-ON-TOP colony formation assay in both ER+ and HER2+ cells, 3) INK128 dose- and time-dependently blocked downstream effectors of mTOR1/2 pathway i.e. p-AKT (at Ser473), p-p70S6K, p-S6 ribosomal protein, p-4EBP1 (interestingly, potent inhibition was also observed in cell line resistant to trastuzumab), 4) INK128 also blocked AKT phosphorylation at Thr308 (although in lesser extent than at Ser473), 5) rapalog-mediated AKT activation was blocked when cells were treated with INK128, 6) in ER+ cell lines INK128 more efficiently blocked p-4EBP1 than rapalog, 7) HER3 expression was significantly upregulated following treatment of trastuzumab-resistant cells (BT474HR) with INK128, and 8) integrin-dependent cell migration was significantly abrogated with INK128 in association with inhibition of RAC1-GTP.

Conclusion: Our preclinical in vitro data demonstrate that concurrent inhibition of mTOR1 and mTOR2 is likely to be more effective in both ER+ and HER2+ breast cancer cells than the inhibition of mTOR1 alone. We are currently studying the effect of INK128 on downstream apoptotic signals in ER+ and HER2+ cell lines, the results of which will be presented in the meeting.

INK128 was generously supplied by InKellikine, San Diego, CA.

In Vitro Potency of mTOR Kinase (TOR1/TOR2) Inhibitor, INK128 in ER+ and HER2 Overexpressing Breast Cancer Cells.

De P, Sun Y, Dey N, Leyland-Jones B. Emory University, Atlanta, GA

Background: Persistent activation of the PI3K-AKT-mTOR1/2 signaling pathway drives aberrant cell growth and proliferation in a variety of tumor types including breast tumors. Recent works have demonstrated a strong association between mutational activation of PIK3CA or loss of function of PTEN and resistance to therapies targeted against the ER or HER2 pathway.

Purpose: Since PI3K-AKT-mTOR is one of the major signaling pathways responsible for the progression of breast cancer, as well as appears to be upregulated during development of the resistance to the endocrine and trastuzumab treatment, suppression of this pathway by administration of INK128 (mTOR1/2 kinase inhibitor) may therefore be efficacious in ER+ and HER2+ breast cancer models.

Experimental Design: To examine this possibility, INK128 was treated in ER+ (MCF7, BT483, T47D and MDA-MB-415), trastuzumab-sensitive HER2+ (BT474), trastuzumab-resistant (BT474HR), and PIK3CA mutated/HER2+ breast cancer cells (HCC1954 and UACC893). We have tested the effects of INK128 on the, (a) cell survival/proliferation, (b) integrin-directed cell migration, and small GTP-ase activation, (c) downstream signaling pathways for proliferation and apoptosis, and (d) 3D ON-TOP-colony formation.

Results: Treatment of ER+ and HER2+ cell lines with INK128 in vitro showed that 1) cell lines were sensitive to single agent INK128 with 50% inhibitory concentration ranging from 15 nM-10 µM in ER+ cell lines and 20 nM -50 nM in HER2+ cell lines, 2) 70-80% anti-proliferative activities were observed on 3D-ON-TOP colony formation assay in both ER+ and HER2+ cells, 3) INK128 dose- and time-dependently blocked downstream effectors of mTOR1/2 pathway i.e. p-AKT (at Ser473), p-P70S6K, p-S6 ribosomal protein, p-4EBP1 (interestingly, potent inhibition was also observed in cell line resistant to trastuzumab), 4) INK128 also blocked AKT phosphorylation at Thr308 (although in lesser extent than at Ser473), 5) rapalog-mediated AKT activation was blocked when cells were treated with INK128, 6) in ER+ cell lines INK128 more efficiently blocked p-4EBP1 than rapalog, 7) HER3 expression was significantly upregulated following treatment of trastuzumab-resistant cells (BT474HR) with INK128, and 8) integrin-dependent cell migration was significantly abrogated with INK128 in association with inhibition of RAC1-GTP.

Conclusion: Our preclinical in vitro data demonstrate that concurrent inhibition of mTOR1 and mTOR2 is likely to be more effective in both ER+ and HER2+ breast cancer cells than the inhibition of mTOR1 alone. We are currently studying the effect of INK128 on downstream apoptotic signals in ER+ and HER2+ cell lines, the results of which will be presented in the meeting.
TRAIL on CSCs derived from CRL-2335 and MDA-MB-468 TNBC

and BSI-201), paclitaxel, docetaxel, cisplatin and cisplatin plus breast tumors. We studied the effects of PARP inhibitors (AZD2281 stem cells compared to ER-positive, PR-positive and HER2-negative Results: TNBC tumors express relatively higher levels of cancer evaluated using Nod/Scid mice. Cancer stem cell levels in human nondifferentiated conditions. Apoptosis was assessed using the Cell ability to form mammospheres, are generated in nonadherent and positive population by fluorescence-activated cell sorting. CSCs Materials and Methods: Cancer stem cells were identified by ALDH- CSCs leading to tumor recurrence.

bulk of tumor cells, but it has a minimal effect on cancer stem cells despite initial response to neoadjuvant chemotherapy. The aim of this study was to determine whether current drug treatment(s) eliminates because of the loss of target receptors such as ER, PR and HER-2 have a worse prognosis compared with other breast cancer subtypes. Hormonal or Herceptin-based therapies were found to be ineffective for the loss of target receptors such as ER, PR and HER-2 amplification. TNBC has increased recurrence and poor survival, despite initial response to neoadjuvant chemotherapy. The aim of this study was to determine whether current drug treatment(s) eliminates bulk of tumor cells, but it has a minimal effect on cancer stem cells (CSCs) leading to tumor recurrence. Materials and Methods: Cancer stem cells were identified by ALDH-positive population by fluorescence-activated cell sorting. CSCs ability to form mammospheres, are generated in nonadherent and nondifferentiated conditions. Apoptosis was assessed using the Cell Death Detection ELISAPLUS kit. Xenograft tumor formation was determined by western blot analysis. Mode of action was explored by cell cycle FACS-analysis. Cleavage of PARP was explored by Western blot. Cytotoxic efficacy was evaluated by MTT assay. Additionally it was explored if inhibition of classical apoptosis could abrogate the effect of AEZS-131.

Results: The level of phosphorylated AKT (pAKT) was reduced when MCF7 cells were treated with AKT inhibitor as well as PI3K inhibitor. However, compensatory AKT activation was observed when these cells were treated with BRAF or MEK inhibitor. KPL4 cells also showed reduction of pAKT when treated with Lapatinib, AKT inhibitor or PI3K inhibitor. Compensatory hyper-phosphorylation of ERK (pERK) was observed in both cell lines and KPL4 xenografts with AKT inhibitor treatment while PI3K inhibition did not induce hyper-ERK phosphorylation. Reduction of pERK level was observed when both cell lines were treated with MEK inhibitor. Downstream analytes like PRAS40 and RPS6 summarize PI3K/AKT pathway activity and correlate well with response to treatment whereas elf4e is a good final readout for activity in the RAS/RAF/MEK/ERK pathway. Treatment with single agents often even shows adverse profile shift through feedback or pathway cross-talk. The comparative analysis of pathway modulation in BCA models to other tumor types in response to drug treatments revealed several adaptive drug resistance mechanism with different oncogenic backgrounds. Discussion: Evaluation of drug specific pathway modulations in cancer cells provided comprehensive information on efficacy of specific agents on target protein and pathway inhibition. Multiplexed pathway analysis provides valuable information for drug resistance mechanisms due to either redundant pathway activation, cross-talk or through feedback mechanism and may guide appropriate selection of targeted drug-combinations or drug-sequencing in clinical setting. For example, the observed increase in ERK phosphorylation due to AKT inhibition could be blocked by combination therapy with a MEK inhibitor.

P3-18-06


Introduction

Overexpression of MAPK has been detected in 34 % of TNBC and has been found to be associated with anthracycline resistance (1). AEZS-131 is a highly selective orally active ERK1/2-inhibitor, which has shown in vivo activity (first in class) in colon cancer. The following study explores mode of action and efficacy in models of TNBC.

Study design

AEZS-131 was tested in a kinase panel of 36 STK, 26 TK and 7 PIK to check for selectivity. Inhibition of Rsk-phosphorylation (cellular substrate of ERK) was evaluated by western blot analysis. Mode of action was explored by cell cycle FACS-analysis. Cleavage of PARP was explored by Western blot. Cytotoxic efficacy was evaluated in 5 TNBC, of which 2 had mutations in the MAPK signal transduction pathway lines, by MTT assay. Additionally it was explored if inhibition of classical apoptosis could abrogate the effect of AEZS-131.

Results

AEZS-131 selectively inhibited ERK with an IC50<4nM. Phosphorylation of Rsk-1 the cellular substrate of ERK was inhibited with an IC 50 of 158 nM. AEZS-131 induced cell cycle arrest in G1 dose-dependently and cleavage of PARP. EC 50 was 0,739 µM for MDA-MB-231 (Ras-mutated), 0,137 µM for MDA-MB-435s (B-RAF mutated), 17,5 µM for MDA-MB-468 (PTEN loss), 25 µM for HCC 1937. HCC 1937 were not inhibited by the compound. Of note, inhibition of classical apoptosis did not alter the cytotoxic effect of the compound in MDA-MB-468 and HCC-1806 cells.

Conclusions

AZS-131 was shown to selectively inhibit ERK at low nM concentrations and to induce G1-arrest. Accordingly, the cytotoxic effect most pronounced in TNBC cell lines with mutations in the MAPK pathway. If classical apoptosis is inhibited, cytotoxic effects remain unchanged, suggesting that AEZS-131 can also induce non-
classical forms of programmed cell death. AEZS-131 should be further explored in TNBCs with overexpression of MAPK or mutations in the MAPK-pathway.

*Äterna Zentaris GmbH, Frankfurt/M, Germany.

(1): MAPK overexpression is associated with antracycline resistance and increased risk for recurrence in patients with triple-negative breast cancer.


P3-18-07
Multiplex RTK Inhibitor Screening Utilizing a Plate-Based Immunoassay with Near-Infrared Detection.
Finkel DJ, James AE, Felix RA, Wegner GJ. R&D Systems Inc., Minneapolis, MN; Toceis Bioscience, Bristol, United Kingdom
Receptor-tyrosine kinases (RTKs) are transmembrane proteins that have been implicated in various cancers and are considered therapeutic targets. The phosphorylation of RTKs on tyrosine residues leads to their activation. We have previously used the Proteome Profiler™ 96 Human Phospho-RTK Antibody Array, a plate-based multiplex immunoassay, to determine the phospho-tyrosine profile of RTKs in MDA-MB-453 breast cancer cells. Most notably, the ErbB family of RTKs were shown to be hyperactivated in this cell line. In this study, we use the same multiplex assay to screen a small molecule kinase inhibitor library (TOCRIS, Catalog # 3514) to identify inhibitors with selectivity towards the ErbB receptors. MDA-MB-453 cells were treated with the kinase inhibitor library prior to treatment with ligands to stimulate ErbB receptor tyrosine phosphorylation and subsequently lysed. The lysates were analyzed with the Proteome Profiler 96 Human Phospho-RTK Antibody Array using near-infrared fluorescent detection. Ligand-dependent tyrosine phosphorylation of all four ErbB receptors was monitored simultaneously and the effects of different kinase inhibitors were determined. While several known ErbB inhibitors blocked ErbB phosphorylation, our screen also identified several inhibitors reported to be selective for other kinases that effectively inhibited ErbB activation. The activation of ErbB2 was selectively inhibited by NH125, GF 109203X, SB 202190, and ligands that effectively inhibited ErbB activation. The activation of ErbB2 was selectively inhibited by NH125, GF 109203X, SB 202190, and ZM 306416 hydrochloride. The results obtained with this multiplex assay were confirmed by singleplex ELISA for ErbB receptors. Hence, the data collected with our multiplex assay provides a rapid method for the analysis of inhibitor effects on a defined signaling pathway. This may facilitate a faster identification of selective kinase inhibitors that have therapeutic potential for treating ErbB-overexpressing cancers.

P4-01-01
Preclinical and Clinical Studies of Estrogen Deprivation Support the PDGF/Ab1 Pathway as a Novel Therapeutic Target for Overcoming Resistance.
Wegel MT, Banerjee S, A'Hern R, Arnedos M, Ghazoui Z, Dumbier AK, Dowsett M, Martin L-A. Institute of Cancer Research, London, United Kingdom; Royal Marsden Hospital, London, United Kingdom
Aim: To determine the relevance of PDGF/Ab1 signaling pathway as a therapeutic target in endocrine resistance in vitro and in vivo.
Rationale: Targeting estrogen stimulation reduces mortality from ER-positive (ER+) breast cancer (BC) but resistance remains a major clinical problem. To identify the molecular mechanisms associated with resistance to estrogen-deprivation, we previously assessed the temporal changes in gene expression during adaptation to long-term culture of MCF7 human breast cancer cells in the absence of estradiol (E2) (LTED), modeling resistance to an aromatase inhibitor (AI). Platelet-derived growth factor (PDGF)/Ab1 signaling was the top adaptive pathway at the point of resistance (p=1.15 E-07), but there is limited evidence for it playing a role clinically.
Methods: Gene expression data from postmenopausal women with ER+ BC at pre-treatment and at 2-week on-treatment with neoadjuvant anastrozole were interrogated. Immunohistochemical staining using antibodies against PDGFRα, β and Ab1 and Ki67 was assessed in paired clinical specimen of 45 patients who had received an AI and presented progressive disease. Proliferation assays, immunoblots and ChIP assays were carried out using either the PDGFR/Ab1 inhibitor nilotinib or siRNA knockdowns in LTED and wild-type MCF7 cells.
Results: In vitro: PDGFRβ and Ab1 expression and phosphorylation were elevated in LTED cells. Cell proliferation was decreased in LTED cells by siRNA knock-down of either PDGFRβ (50% p=0.01), Ab1 (40% p=0.01) or the combination (70% p=0.01). Inhibition of Ab1 activity using nilotinib decreased ER protein levels and suppressed ER-mediated transcription by reducing recruitment of AIB1 and CBP to the promoter of E-responsive genes TFF1 and GREB1. Clinical: Data from 81 patients treated with an AI in the neoadjuvant setting, showed increases in PDGFRα gene expression after two weeks (1.25 fold, p=0.003). Low PDGFRα at baseline was associated with a better response. Of note, paired pre-AI-treatment and relapse clinical specimens from a cohort of patients treated in the primary or advanced setting revealed increases in tumor protein expression of PDGFRα (1.39 fold, p=0.0065), PDGFRβ (4.32 fold, p=0.006) and Ab1 (1.8 fold, p=0.001) at the point of relapse. Tumor (T) and stromal (S) expression of PDGFRα as well as PDGFRβ was significantly correlated in pre-treatment and relapse samples (Table 1).

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High post-treatment tumor and stromal PDGFRβ levels were associated with a short time to treatment failure (TTF) (T: Rs -0.284, p=0.066; S: Rs -0.307, p=0.046). Expression of PDGFRα in relapsing tumor specimens was correlated with Ab1 expression (Rs 0.342, p=0.027) and Ki67 levels (Rs 0.39, p=0.01). Furthermore, changes in Ab1 correlated significantly with changes in ER expression (Rs 0.307, p=0.048).
Discussion: These in vitro and clinical data support a biological interaction between PDGFR/Ab1 and ER-signaling and suggest that the PDGFR signaling pathway warrants clinical evaluation as a therapeutic target in endocrine resistant breast cancer.

P4-01-02
Morrison GD, Fu X, Ithimakin S, Rimawi MF, Wicha MS, Osborne CK, Schiﬀ R. Baylor College of Medicine, Houston, TX; University of Michigan, Ann Arbor, MI
Background: Endocrine therapy is the most effective therapies for estrogen receptor positive (ER+) breast cancer, but resistance is still a major clinical problem. Tumor initiating cells (TICs) in drug resistant tumors are believed to be part of the contributing factors for tumor relapse. Here we focused on the TICs and how they are related to HER2 expression and its downstream factors in acquired endocrine resistance models.
Materials and Methods: We have established a panel of endocrine resistant cell models through long-term treatment with tamoxifen (Tam, 10^-7 M, >6 months). Resistance to tamoxifen treatment in cells (TamR) was conﬁrmed by growth curve assays (Celigo, Cyntellect...
Inc., San Diego, CA). In vitro mammmosphere assays were applied to assess the mammosphere forming ability of parental and resistant cells. Aldefluor assays (StemCell Technology) were performed to measure the aldehyde dehydrogenase (ALDH-1) expressing cell population as a marker of TICs. Flow analysis was performed to evaluate HER2 expression in the ALDH subpopulations of both parental and TamR cells. Immunofluorescence staining was further used to detect the expression of ER, HER2, phosphorylated STAT3, and β-catenin in sorted ALDH+/− subpopulations.

Results: We found that endocrine resistant cells have a higher mammosphere forming ability than their parental counterparts. In addition, we showed that the ALDH+ subpopulation was significantly upregulated in all TamR derivative lines.

Further analysis of the ALDH populations indicated that HER2, as well as downstream survival factors such as pSTAT3s727 and β-Catenin, are upregulated in the ALDH+ populations as compared to the ALDH-population, especially in TamR derivatives.

Discussion: Our data suggest that deregulated HER or other growth factor receptor signaling can potentially lead to the enrichment of TICs as a potential contributor to endocrine resistance. We will further investigate the role and molecular signaling of TICs in endocrine resistance of breast cancer, as well as whether and to what extent more potent anti-HER inhibitors can improve endocrine sensitivity and circumvent resistance.

P4-01-03
Establishment and Characterization of an Endocrine Resistance Model In Vitro and In Vivo by Inducible PTEN Knockdown.
Fu X, Shea M, Biswal NC, Mitchell T, Giuliano M, Healy NA, Meerbrey KL, Joshi A, Westbrook T, Hilsenbeck SG, Osborne CK, Schiff R. Baylor College of Medicine, Houston, TX; National University of Ireland, Galway, Ireland

Background: Growth factor receptor and estrogen receptor (ER) are two major driving pathways for initiating and sustaining breast cancer (BC) development and progression. We have previously shown that an inverse correlation exists between the PI3K pathway and ER expression/activity in luminal type BCs. High PI3K activation signaling correlates to the luminal B subtype of BC with low ER expression/activity. However, the involvement of the PI3K pathway tumor suppressor PTEN in resistance to endocrine therapy is less clear. Here we attempt to develop an experimental system to better understand the role of PTEN in this resistance.

Materials and Methods: Two luminal BC cell lines, MCF7L and ZR75-B, were stably infected with a lentivirus pINDUCER (Meerbrey et al., PNAS, 2011), containing Tet-on responsive shPTEN, turboRFP (tRFP) as an inducible tag, and enhanced GFP (eGFP) as a constitutive expressed tag for positive cells selection. Immunoblotting of PTEN, phosphorylated (p) Akt, pMAPK, pc-Jun, ER, and ER’s downstream gene products (PR, IGF-1R) was performed on cells after 6 days of growth factor receptor and estrogen receptor (ER) signaling correlates to the luminal B subtype of BC with low ER expression/activity. However, the involvement of the PI3K pathway tumor suppressor PTEN in resistance to endocrine therapy is less clear. Here we attempt to develop an experimental system to better understand the role of PTEN in this resistance.

P4-01-04
Effects of CYP2D6 Phenotype and Drug Adherence on Tamoxifen Metabolite Levels.
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Background: Although the activity of cytochrome P450 2D6 (CYP2D6), the enzyme responsible for conversion of tamoxifen (TAM) to its most important active metabolite endoxifen, varies significantly with genotype. Routine genotype testing in patients on TAM has recently been discouraged. Conflicting results in publications regarding the prognostic utility of this test remain unexplained. Confounding factors could be lack of predicted correlation between CYP2D6 genotype and TAM active metabolites, or variability of patient compliance.

Methods: Consecutive breast cancer patients on TAM were asked to enroll in a study to examine the relationship between CYP 2D6 phenotype, patient-reported treatment adherence, and TAM metabolites levels. Patients were genotyped for CYP2D6 polymorphisms using long-range PCR allele-specific amplification and single-nucleotide primer extension assay. From the genotypes, four phenotype groups were defined: Ultra rapid Metabolizer (UM), Extensive Metabolizer (EM), Intermediate Metabolizer (IM) and Poor Metabolizer (PM). Plasma was collected after at least 6 weeks of TAM (20 mg daily). The parent drug TAM, as well as 4-hydroxy N-desmethyl tamoxifen (endoxifen), 4 hydroxy tamoxifen (4OHtam) and N-desmethyl-tamoxifen (NDtam), were determined by Liquid Chromatography tandem mass-spectrometry (LC-MS/MS). Patients also completed a questionnaire about ethnicity, side effects, concurrent medications and tamoxifen adherence. Correlation between plus and minus Dox groups under E2, Tam, or Ful treatment is ongoing.

Discussion: These data further support the existence of crosstalk between PI3K and ER pathways in luminal type BC. Decreasing PTEN levels by shRNA renders the luminal type BC cells de novo resistant to endocrine therapy both in vitro and in vivo. The pINDUCER PTEN knockdown system combined with live animal imaging offers successful real-time, noninvasive tracking of endocrine sensitivity by controllably manipulating the level of target gene. Combination therapies to overcome endocrine resistance under PTEN knockdown conditions are currently underway.
between metabolite/TAM ratio and phenotype was tested by Spearman correlation test. Relationship between metabolite levels and adherence was tested by Wilcoxon rank sum test. Chi square test was used to compare proportions.

**Results:** Of the 100 patients enrolled there were 62 Caucasians, 25 Asians, 4 Africans and 6 Unknown. We found a strong correlation between ratio of endoxifen/TAM and phenotype (p <0.001) (Table 1) Over a 2 week period 68 never missed a TAM dose, 25 missed 1-2 times, 2 missed 3-5 times and 2 >5times (2 missing data). In EM group we found significantly lower levels of TAM (p <0.001), NDtam (p=.008), 4OHtam (p=.003) in less adherent patients. A trend to decreased levels was also shown for endoxifen (p=.081). No associations were found between adherence or phenotype activity and side effects.

**Conclusions:** Our data suggests the predicted association between endoxifen levels and genotype. However, non-adherence may have a significant confounding effect. Prospective studies to evaluate the prognostic impact of CYP2D6 variants for patients on adjuvant tamoxifen should be done but results could be confounded by variable drug adherence if this is not measured concurrently.

**P4-01-05**


**Background:** Resistance to antiestrogens is a major problem in metastatic breast cancer. Preclinical data suggest that growth factor signaling and angiogenesis may promote endocrine resistance and blocking such pathways may delay resistance.

**Methods:** We conducted a phase II clinical trial of adding sorafenib, a VEGFR and Ras/Raf/MAPK inhibitor, to antiestrogen therapy in patients with metastatic ER-positive breast cancer. Patients were required to have progressive disease on an anstiestrogen or have maximum response with residual measurable disease. A core biopsy of accessible breast disease was offered at entry and after 28 days of sorafenib. Microarray experiments were performed on frozen tissue obtained from 4 paired biopsies using Affymetrix Gene Chip HG-U133 Plus 2. Data was normalized based on the GCRMA method and Expression values of each gene were analyzed based on the significant analysis of microarray (SAM) method for paired data. Gene set analysis on KEGG pathways was performed using the gene set enrichment analysis (GSEA) method, where genes were ranked based on the absolute values of their SAM test statistics. Serum was collected on day 1 and day 28 for biomarker comparison using ELISA. Primary study endpoint was response rate by RECIST criteria after 3 months of sorafenib and secondary endpoints were safety, time to progression (TTP), and biomarker assessment.

**Results:** Planned sample size was 43 but the study closed after 11 patients because of slow accrual. Median age was 45 years (Range 39-72). 7 patients were on tamoxifen, 3 on an aromatase inhibitor, and 1 on fulvestrant. Of the 11 patients enrolled, 8 had progressive disease (PD) on entry and 3 had confirmed stable disease (SD) on antioestrogen alone. One patient with SD at entry discontinued sorafenib after 2 weeks because of a grade 3 rash. Of the 10 patients evaluable for response, 7 had SD (70%) and 3 had PD. Median TTP after adding sorafenib was 182 days (6 months) and in the 8 patients who entered the study with PD, 5 converted to SD (62%) with a median TTP of 192 days (6.4 months). One patient remains on treatment after 16 months of enrollment. Most common adverse events were rash in 9 patients, weight loss in 8 and hypertension in 6. Hypophosphatemia was seen in 11 patients, hypokalemia in 9, and elevated ALT/AST in 4. The majority of toxicities were grade 1. There were 6 grade 3 toxicities; rash, anorexia, hypokalemia, colitis, and 2 hypophosphatemia. No grade 4 toxicities occurred. Microarray analysis identified 29 enriched pathways with a false discovery rate of less than 25%. There was a significant reduction in mean serum Transforming growth factor beta (TGF-β) and platelet derived growth factor receptor alpha (PDGFRα) on day 28 (P values =< 0.0001 and 0.017, respectively). Tissue and serum biomarker correlates will be presented in detail at the meeting.

**Conclusions:** Sorafenib can overcome resistance to antiestrogens, particularly tamoxifen, and may help avoid the routine early use of chemotherapy for endocrine resistant disease. Further study of strategies to overcome endocrine resistance is warranted to help preserve patient quality of life and to investigate mechanisms of resistance.

**P4-01-06**

Global Characterization of the SRC-1 Transcriptome Identifies Disintegrin C as an ER-Independent Mediator of Endocrine Resistant Breast Cancer. 
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**Background:** The development of breast cancer resistance to endocrine therapy results from an increase in cellular plasticity leading to the development of a steroid independent tumour. The p160 steroid coactivator protein SRC-1 drives this tumour adaptability. SRC-1 is central to the development of the endocrine resistant phenotype and is an independent predictor of disease free survival in tamoxifen-treated patients. Here, using discovery studies we identify Disintegrin C (DC), a multidomain transmembrane glycoprotein as a direct, ER-independent target of SRC-1.

**Methods:** SRC-1-ChIP sequencing was performed in endocrine resistant (LY2) cells. Microarray experiments were performed on LY2 cells transfected with scrambled siRNA or SRC-1 siRNA, +/- 4-OHT to assess SRC-1-dependent gene expression. Combining the ChIP-seq and expression array datasets, a total of 2,065 genes were significantly downregulated (p<0.05) following SRC-1 knockdown. DC transcript levels were measured following knockdown and over-expression of SRC-1 and ERα. Mouse xenograft and SRC-1 knockout models were used to examine DC expression in vivo. A TMA comprising 560 patients was stained for DC. Functional validation studies were performed using 3D culture and migration assays, with transient knockdown of DC and treatment with JC1, an inhibitory peptide against DC.

**Results:** Treatment with 4-OHT significantly increased the number of ChIP-enriched intervals identified. The majority of peaks in the 4-OHT treated sample were close to the transcriptional start site, in the promoter, first exon or upstream of the promoter. As a defined
ER coactivator, an overlap between ER binding sites and those identified for SRC-1 is expected. 43% of high confidence SRC-1 peaks in 4-OHT-treated lymphocytes contained an ERE binding motif. This raises the possibility that, in endocrine resistance, SRC-1 can drive transcription independent of ER. Treatment with 4-OHT resulted in substantial recruitment of SRC-1 to the DC promoter. In primary tumours there was a significant association between transcript levels of SRC-1 and DC. Cells derived from mammary tumours of the SRC-1+/−/PyMT mouse lacked DC, but there was high expression in the wild type PyMT mouse. Knockdown of ERα in LY2 cells did not alter DC expression. Knockdown of DC reduced cell migration and restored cell differentiation in resistant cells. In xenograft studies treatment with 4-OHT increased tumour volume in the resistant tumours and induced DC expression. Treatment with recombiant JCl1 reduced cellular migration in endocrine resistant cells. Kaplan-Meier estimates of disease free survival show that DC is a strong predictor of disease free survival in breast cancer (p<0.0001). SRC-1 and DC are significant independent predictors of disease recurrence (odds ratios 2.10 and 2.44).

Conclusion: Our discovery studies have uncovered a steroid independent SRC-1 mediated network in endocrine resistant breast cancer. We identified a new SRC-1 target, DC. DC represents a rational new therapeutic target with a robust companion biomarker for treatment of endocrine resistant tumours.

Results: Expression of Hhg signaling molecules and the targets are significantly activated in OHTR cells resistant to 0.5 and 1μM tamoxifen. Serial passage of the resistant cells in mice resulted in aggressive transformation of tumors that micro-metastasized to lung and liver. Concurrent increase in Hhg marker expression, TICs and acquisition of epithelial mesenchymal transition were observed in the cells derived from these tumors. Depletion of GLI1 in OHTR cells resulted in reduced proliferation, colony formation and mamsphere formation and increased apoptosis in presence of tamoxifen. Treatment of OHTR cells with GDC-0449 and tamoxifen inhibited cell proliferation in vitro and supressed tumor growth and distant metastasis of tamoxifen resistant xenografts in mice. Primary human breast tumors expressing higher level of GLI1 demonstrated a trend towards increased risk of recurrence (p=0.08) after tamoxifen therapy.

Conclusions: Our work has demonstrated for the first time that activated Hhg signaling is an alternate survival mechanism for tamoxifen resistant breast cancer. Furthermore, targeting these tumors with a combination of tamoxifen and anti-Hhg therapy inhibited tumor growth and development of distant metastases demonstrating efficacy of this approach. Clinical trials using this combination in patients with advanced ER+ breast cancer are warranted and are under development.

P4-01-08
The Role of Ser991 PELP1 Phosphorylation in Therapy Resistance and Metastasis of Breast Cancer.
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BACKGROUND: Growth factor induced activation of MAPK pathway is suggested to play a critical role both in invasive and hormone therapy resistant breast cancer via ligand independent activation of ERα. Proline Glutamic acid Leucine Rich Protein (PELP1) is a well established ER-coregulator that plays a critical role in ER’s nuclear and extra-nuclear functions. PELP1 functions as a proto-oncogene and deregulation of PELP1 expression is linked to increased proliferation, metastasis and therapy resistance. The objective of this study is to examine, whether growth factor signaling modulates PELP1 mediated ER coactivation functions via MAPK pathway.

METHODS: Bioinformatics and phosphopeptide specific antibodies were employed in this study. In vitro kinase assays and MAPK inhibitors were used to confirm MAPK phosphorylation of PELP1. Site directed mutants and ERE reporter gene assays were used to demonstrate the significance of MAPK site in PELP1. Growth factor and estrogen regulation of PELP1 phosphorylation at Ser991 was validated in therapy sensitive (MCF7, ZR75) and resistant (MCF-7 HER2, MCF7-Tam and MCF-7Ca-LTLT cells using PELP1 specific phosphoantibody. Breast cancer model cells stably expressing PELP1 mutant or PELP1 peptides encompassing MAPK phosphorylation site were used to test the significance of this MAPK site in PELP1 using proliferation, migration, and invasion assays. IHC analysis using PELP1 phospho antibodies was performed using murine xenograft tumors and human breast tumor arrays (TMA).

RESULTS: Growth factors (EGF, Heregulin) induced Ser991 phosphorylation of PELP1 in murine mammary epithelial cells and also in human breast cancer cell lines. Growth factor mediated Ser991 PELP1 phosphorylation was abrogated by ERK1/2 MAPK pathway inhibitors including PD98059 and U0126. In ERE-reporter luciferase assays using ZR75 cells, PELP1 (WT) but not the ser991Aa mutant of PELP1 corroborated growth factor-ER crosstalk. Therapy resistant model cells (MCF-7-Tam, MCF-7-LTLT-ca and MCF-7...
HER2) exhibited enhanced Ser991 PELP1 phosphorylation compared to parental MCF7. TAT-Peptide encompassing Ser991 inhibited growth factor-mediated phosphorylation of PELP1 and revealed a potent ability to inhibit the growth of therapy resistant cells. Xenograft tumors of hormone therapy model cell lines showed increased Ser991 PELP1 phosphorylation and enhanced nuclear localization compared to hormonal sensitive xenograft tumors. IHC analysis of human breast invasive TMA revealed enhanced PELP1 ser991 phosphorylation in advanced metastatic tumors with prominent nuclear/nucleolar localization compared to normal and benign tumors.

CONCLUSIONS: In this study, we identified that growth factor-MAPK pathway modulate ER coregulator PELP1 functions via phosphorylation and demonstrated that Ser991 phosphorylation of PELP1 has the potential to govern the growth Factor-ER cross talk leading to therapy resistance and metastatic phenotype of breast cancer. Peptides encompassing Ser991 PELP1 site and Phospho-antibodies targeting Ser991 represent a novel therapeutic target and diagnostic markers in breast cancer. Supported by DOD Fellowship W81XWH-09-1-0010 (BCN) and DOD W81XWH-08-1-0604 (RKV)

**P4-01-09**

**Reduced CYBA Expression Leads to the Development of Fulvestrant Resistance in a Breast Cancer Cell Line.**

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**Background:** Acquired resistance to endocrine therapy is an important problem in the management of advanced breast cancer. In this study we used a cell line model to investigate the evolution of fulvestrant resistance. We examined gene expression and promoter methylation in estrogen-dependent MCF7 cells and compared two derived cell lines, estrogen-hypersensitive LCC1 and fulvestrant-resistant LCC9, using array technology. Thus we determined the epigenetic changes associated with the evolution of estrogen independence and fulvestrant resistance, which revealed a role for CYBA/p22phox in the acquisition of resistance.

**Material and Methods:** Estrogen-dependent gene expression was measured in MCF7, LCC1 and LCC9 cells using Illumina HT-12 microarrays. In parallel, differential methylation between MCF7, LCC1 and LCC9 cells was examined using Illumina Infinium methylation 27 arrays.

**Results:** Despite marked physiological differences between MCF7, LCC1 and LCC9 cells there were few genes which were both differentially methylated and differentially expressed. However, CYBA was underexpressed in LCC1 (p<10^-5) and LCC9 (p<10^-4) and had a methylated promoter in LCC1 (p<10^-5) and LCC9 (p<10^-5), with reduced expression correlated to increased estrogen independence. This gene codes for the protein p22phox, a critical component of membrane NADPH oxidase. We verified expression by qRT-PCR and Western blot, and methylation by CpG island bisulphite sequencing, demonstrating significant and biologically relevant changes. Fulvestrant-resistant LCC9 cells exhibited a 20-fold reduction in CYBA expression compared to MCF7, corresponding to undetectable levels of p22phox. Due to its role in the generation of reactive oxygen species (ROS), we investigated the cellular response to oxidant damage, finding that LCC9 cells showed reduced sensitivity to oxidant damage when compared to MCF7 (p<10^-5), in keeping with impaired NADPH oxidase function. We then treated MCF7 cells with a ROS scavenger, N-acetyl cysteine, which reduced their sensitivity to fulvestrant (p<10^-13). Using siRNA, we knocked down CYBA expression, which conferred slight fulvestrant resistance on MCF7 cells, mitigating fulvestrant-induced growth arrest (p<10^-7).

**Discussion:** The striking differences in methylation in the CYBA promoter and gene expression between these cell lines lead us to conclude that the repression of this gene represents an important step in the development of fulvestrant resistance. As these cells develop estrogen independence and fulvestrant resistance, CYBA/p22phox levels drop, making the cells more resistant to oxidant damage caused by estrogen deprivation and, ultimatey, providing a selective advantage against fulvestrant-induced growth arrest and cell death. CYBA/p22phox may represent a novel druggable target in breast cancer, either in the prevention of the acquisition of fulvestrant resistance, or the further treatment of resistant tumours.

**P4-01-10**

**The Role of the Steroid Receptor Coactivator SRC1 and Its Functional Partner HOXC11 in the Development of Endocrine Resistant Breast Cancer.**

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**Background:** The steroid receptor coactivator; SRC1, has been well described in the development of endocrine resistant breast cancer. SRC1 associates with clinically aggressive tumours and promotion of distant metastasis. It directly interacts with the developmental transcription factor, HOXC11 and together they are found to strongly predict poor disease-free survival in breast cancer patients (hazard ratios: 5.79; P < 0.0001). In this study, we investigate the mechanism of SRC1 and HOXC11 action in tumour adaptability and subsequent resistance to endocrine therapy.

**Materials and Methods:** Cells which are resistant to tamoxifen (LY2s) have enhanced SRC1 and HOXC11 mRNA and protein expression in comparison to their endocrine sensitive parent cells (MCF-7s). ChIP-sequencing data for SRC1- and HOXC11- DNA interactions in conjunction with DNA microarray and RNA-sequencing data identified potential signalling targets at play in the LY2 model of endocrine resistance. Real-time analysis and flow cytometry confirmed these interactions at a transcriptional and protein level. These observations were further confirmed in primary breast cancer cultures using flow cytometry.

**Results:** SRC1 and HOXC11 interactions are driven in tamoxifen treated LY2 resistant cells. Combined SRC1 ChIP-sequencing and expression array data analysed in conjunction with HOXC11 ChIP-sequencing and RNA-sequencing data reveal that the SRC1/ HOXC11 transcriptional process can orchestrate the loss of luminal cell markers such as ERα, CD24 and PTCH1 whilst concomitantly upregulating mediators of tumourigenicity such as CD44 and MSI2. Primary breast cancer cultures confirm the loss of CD24 in tamoxifen resistant patients. In these patients, loss of CD24 was accompanied by loss of steroid receptor expression (ERα and PR) and by a gain of the basal marker CD44.

**Discussion:** Here, we describe a new signalling pathway where SRC1 and HOXC11 regulate two distinct but complementary mechanisms to drive tumour adaptability. Silencing of luminal cell markers and a concomitant increase in a basal cell phenotype has the potential to alter the survival mechanism of breast cancer cells to evade targeted therapy.
P4-01-11
Autocrine Human Growth Hormone (hGH) Reduces the Sensitivity of Breast Cancer Cells to Treatment with Tamoxifen and Fulvestrant.

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Despite recent advances in breast cancer treatment regimes, intrinsic or development of resistance to conventional breast cancer therapies is still a significant clinical challenge. Expression of growth factors by tumour cells has been proposed as one mechanism for developing resistance to radiotherapy and chemotherapeutic agents. The growth hormone/insulin-like growth factor-1 axis is emerging as an important mediator of tumour development in human breast cancer. Recent studies have demonstrated that humans with growth hormone receptor deficiency are protected from developing cancer. Studies investigating human growth hormone (hGH) expression in human breast cancer have demonstrated that hGH expression is associated with specific histopathological features and survival outcomes for patients. Increased hGH expression is significantly associated with lymph node metastasis, tumour stage, proliferative index, worse relapse free survival and reduced overall survival in mammary carcinoma. In recent studies we have demonstrated that autocrine hGH promotes cell proliferation, cell survival and oncogenicity of human mammary carcinoma cells, enhancing anchorage independent cell growth, and supporting tumour formation in immunodeficient mice. Autocrine hGH also promotes mammary carcinoma cell migration/invasion and epithelial to mesenchymal transition and tumour vascularisation in xenograft studies. In this study we investigated whether autocrine hGH promotes resistance to anti-estrogen therapeutic drugs, sensitivity to tamoxifen and fulvestrant using the human breast cancer cell lines MCF-7, T47D and BT474. Stable transfection with an expression vector containing the hGH gene enhanced MCF-7, T47D and BT474 cell viability, total cell number and anchorage independent growth following treatment with tamoxifen or fulvestrant when compared to control transfected cells. Conversely, treatment of BT474 cells with an hGH receptor antagonist (B2036), increased BT474 cell sensitivity to treatment with tamoxifen or fulvestrant. Using an estrogen response element (ERE) luciferase assay, we observed that autocrine hGH enhanced basal estrogen receptor (ER)-mediated transcriptional activity. Our results demonstrate that forced expression of hGH in mammary carcinoma cells enhances ER-mediated signal transduction and promotes resistance to tamoxifen and fulvestrant, while functional antagonism of hGH increased cell sensitivity. Consequently, antagonism of the hGH receptor may help maintain sensitivity to anti-estrogen therapy and improve the prognosis of patients with hormone sensitive breast cancer.

P4-01-12
The Unfolded Protein Response (UPR) and Pro-Survival Autophagy Contributes to Antiestrogen Resistance in Breast Cancer.

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In 2011, most of the 192,000 newly diagnosed cases of invasive breast cancer will be estrogen receptor alpha positive (ER+). Endocrine therapy, administered as an antiestrogen, e.g., Tamoxifen (TAM) or Faslodex (FAS; Fulvestrant; ICI 182,780) or an aromatase inhibitor (AI), e.g., Letrozole or Exemestane is the least toxic and most effective means to manage the hormone-dependent breast cancer in such patients. However, resistance to endocrine therapy remains a significant clinical problem. Previously, we have shown that antiestrogen resistant breast cancer cells over-express X-Box Binding Protein 1 (XBP1), a transcription factor that belongs to the basic region/leucine zipper (bZIP) family. XBP1(U) and XBP1(S) variants result from an unconventional splicing of the XBP1 mRNA by IRE1alpha. In the unfolded protein response (UPR), endoplasmic reticulum proteins (IRE1alpha, ATF6 and PERK) sense cellular stress to regulate the accumulation of unfolded proteins. Initially a compensatory mechanism allowing cells to recover normal endoplasmic reticulum function, prolonged UPR may induce cell death; this is often dependent upon which arm of the UPR predominates. XBP1 is an obligate component in both the IRE1alpha and ATF6 arms of the UPR. In this study, we show that in MCF7/LCC9 [FAS resistant; TAM cross-resistant] cells, there is an increase in UPR signaling as detected by increased expression of BiP/GRP78. Transient expression of XBP1(S) in MCF7/LCC1 [antiestrogen sensitive] cells show decreased sensitivity to FAS that correlated with increased levels of both basal and FAS-induced autophagy as measured by cleaved LC3BII protein fragment, GFP-LC3 activity, and reduced expression of p62/SQSTM1. Furthermore, we show that MCF7/LCC9 cells survive by activating pro-survival autophagy through UPR that is regulated by the transcription factor c-MYC. An inhibitor of c-MYC, 10058-F4, synergized with FAS to inhibit proliferation in MCF7/LCC9 cells by preventing pro-survival autophagy and increasing apoptosis. Thus, our study shows that antiestrogen resistant breast cancer cells evade cell death by activating XBP1-mediated UPR that results in c-MYC-mediated prosurvival autophagy.

P4-01-13
Biology of Aggressiveness and Tamoxifen Resistance in Hormone Receptor-Positive Very Young Age Breast Cancer.

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It has been known that young breast cancer patients have worse outcomes compared with older premenopausal or postmenopausal patients. Survival difference between young and older breast cancer patients is evident only in hormone receptor positive breast cancer regardless of tamoxifen treatment according to our previous report. However, the biology of aggressiveness and endocrine resistance of breast cancer in very young women is largely unknown. The purpose of this study is to find molecular characteristics of hormone receptor positive breast cancers of very young age women (<35) compared with those of older premenopausal women.

We extracted mRNA from fresh frozen hormone receptor positive primary breast cancer tissues of 24 young age breast cancer patients (<35 years) and 31 older premenopausal women (40 to 49 years) by standard method. All tumor specimens analyzed contained more than 50% tumor cells. Hormone receptor status was determined by immunohistochemistry (IHC). Gene expression microarray experiment was done in the 55 samples using Illumina HumanRef-8 v3 Expression BeadChip (Illumina, Inc., San Diego, CA). Functional and pathway analysis of differentially expressed genes were done using DAVID (http://david.abcc.unc.edu/home.jsp) and Ingenuity pathway analysis (IPA, http://www.ingenuity.com). Ki-67 assay was done using IHC in 4,957 ER+ breast cancer patients of Seoul National University cohort and 1,863 ER+ patients from National Cancer Center, Korea.
355 genes were upregulated (>1.5 fold) and 209 genes were downregulated in breast cancer tissues of young age patients (<35) compared with those of older patients. In pathway analysis of the highly expressed genes in young patients, cell cycle function and pathway was significantly activated. The genes of central role in this pathway were MYC and CCND1. In IHC assay for 4,957 ER+ Seoul National University dataset, the proportion of high Ki-67 expression (>=10%) was positively correlated with decreasing age: 26.4%, 24.0%, 21.3%, 14.8%, 12.0% of women aged <30, 30-34, 35-39, 40-49, 50-59, respectively (p<0.001). The same correlation pattern between younger age and high Ki-67 expression was also seen in another 1,863 patient cohort.

In conclusion, we showed that genes involved in cell cycle pathway were upregulated in very young age (<35) ER+ breast cancer using microarray study. It was validated in large data set using Ki-67 IHC assay. High proliferation and fast cell cycle could be a mechanism of worse outcome and tamoxifen resistance of ER+ very young age breast cancer patients.

P4-01-14
Fulvestrant Regulates Epidermal Growth Factor (EGF) Ligands and Induces EGF Receptor Activation in MCF-7 Breast Cancer Cells.
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Selective Estrogen Receptor Modulators (SERMs) 4 Hydroxy Tamoxifen (4-HT) and fulvestrant (fulv) inhibit estrogen receptor α (ER) positive breast cancer growth. We have observed that treatment (24 to 48 hours) of fulv in MCF-7 cells induced a 190kDa tyrosine phosphorylated band, which was blocked by EGFR and HER-2 tyrosine kinase inhibitors erlotinib and lapatinib. Immunoprecipitation showed EGFR, HER-2, and HER-3 were all phosphorylated after fulv treatment. 4-HT and estradiol did not cause this phosphorylation. No changes in receptor level after fulv treatment were noted. Downstream MAPK signaling was also blocked by erlotinib and lapatinib. Fulv induced activation of EGFR was ER dependent, since fulv treatment in C4-12, an ER negative cell line derived from MCF-7 cells, did not induce EGFR activation. Co-treatment with estradiol and fulv prevented EGFR activation. To explore the possibility that fulv enhanced ligand expression, we collected conditioned media (CM) from MCF-7 cells after 48 hours of treatment. pEGFR was lost when CM was removed, but recurred within 30 minutes. Cycloheximide abolished the ability of fulv to activate EGFR suggesting autocrine production of EGF ligands was induced by fulv. To detect specific EGFR ligands, we used qPCR to measure various EGFR ligand mRNA levels in MCF-7 and C4-12 cell at 1, 4, 24 and 48 hours after fulv treatment. TGF-α and HB-EGF mRNAs were upregulated over 48 hours, which correlated well with pEGFR activation. 4-HT did not affect mRNA levels of these ligands. In contrast, amphiregulin (AREG) mRNA levels were substantially reduced 48 hours after fulv treatment. A similar trend was seen for AREG mRNA levels in 48HT treated cells but to a much lesser extent. There was no change in any EGF ligand mRNA levels in ER negative C4-12 cells. These qPCR data suggested differential regulation of EGFR ligands by fulv treatment contributed to EGFR family member activation and was dependent on ER expression. Upon fulv treatment, levels of ER were diminished with decreased detection of S167 and S118 ER phosphorylation sites. Monolayer cell growth analysis showed that while fulv treatment in SFM reduced MCF-7 cell numbers compared to control treatment, erlotinib plus fulv significantly reduced cell numbers below the level of cells maintained in fulv alone. In conclusion, we show that fulv, but not E2 or 4-HT, activates EGFR family members accompanied by upregulation of ligands. Since SERM resistance has been associated with EGFR family member activation, differential control of EGFR ligand gene expression by ER may contribute to the development of fulvestrant resistance in breast cancer.

P4-01-15
Alteration of Y-box Binding Protein-1 Expression Modifies the Response to Endocrine Therapy in Estrogen Receptor Positive Breast Cancer.
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Y-box binding protein-1 (YB-1) plays an important role in tumor progression and drug resistance. This study examined whether YB-1 is involved in the alteration of response to endocrine therapy in ER-positive breast cancer cells. MCF7 cells that stably expressed YB-1 (MCF7-YB-1) and vector control cells (MCF7-vector) were established. These cells were used to analyze the expression of the factors related to the ER and growth factor receptor signaling pathways, response to the antiestrogens (tamoxifen and fulvestrant), and estrogen responsive element (ERE) activity. The effect of knocking down endogenous YB-1 expression was tested in wild-type MCF7 cells. In addition, the expression of the YB-1 and the factors related to the ER and growth factor receptor signaling pathways were evaluated in the clinical breast cancers treated with preoperative chemotherapy. The expression of HER2, ALB1, p-Erk and c-Myc were increased in the MCF7-YB-1 cells. In contrast, knocking down of YB-1 decreased the expression of these factors, but increased the expression of ERα in the wild-type MCF7 cells. Furthermore, sensitivity to antiestrogens was decreased in the MCF7-YB-1 in comparison to those in the MCF7-vector cells. In the MCF7-YB-1 cells, the expression levels of p-Erk and c-Myc were continuously upregulated when the cells were treated either with tamoxifen or fulvestrant. The ERE activity was decreased in the MCF7-YB-1 cells in comparison to the MCF7-vector cells, and the ERE activity of the MCF7-YB-1 cells was inhibited by fulvestrant at a lower concentration than that which inhibited the ERE activity of the MCF7-vector cells. In the ER-positive clinical breast cancers treated with preoperative chemotherapy, significantly more of the specimens that showed increased or positive nuclear YB-1 expression after the chemotherapy were positive for HER2 expression. These data suggest that alteration of YB-1 may modify the crosstalk between the ER pathway and HER2 pathway in ER-positive breast cancer cells, and consequently may alter the response to endocrine therapy in these cells.

P4-01-16
The Influence of CYP2D6 Genetic Polymorphisms on Variability of Tamoxifen Metabolism in the Lebanese Breast Cancer Population.
Introduction The selective estrogen receptor modulator tamoxifen is commonly used for the treatment and prevention of breast cancer. Tamoxifen needs to be converted by the highly polymorphic enzyme, CYP2D6, to its active metabolites (4-hydroxytamoxifen and endoxifen) to exert its anti-estrogenic effect. Several clinical trials have shown that CYP2D6 poor metabolizers have decreased benefit from tamoxifen compared to extensive metabolizers. Large ethnic variations have been noticed in the frequency and effect of CYP2D6 polymorphisms.
Objectives To assess for the frequency of CYP2D6*3, *4, *5 (nonfunctional alleles with *5 being a gene deletion), *41 (reduced functional allele) as well as CYP2D6*1N (allele copy number) in a sample of Lebanese participants receiving maintenance doses of tamoxifen for early stage breast cancer, and to assess the effect of these polymorphisms on tamoxifen metabolism in the same subjects.

Methods 111 (median age = 48.7 ± 8.6 years) Lebanese breast cancer women were recruited. Blood for isolation of DNA and measurement of tamoxifen metabolites was collected. CYP2D6 genotyping was performed using real-time PCR. Plasma levels of tamoxifen were being measured using HPLC.

Results The allele frequencies of CYP2D6*3, *4, and *41 were 0.9, 15.9 and 12.1 respectively. 18.9% of patients had more than 2 CYP2D6 copies and 9% had only 1 copy. Tamoxifen plasma concentration didn’t significantly vary among the 4 phenotypic groups, although there was a trend observed whereby PMs had the highest tamoxifen plasma concentrations and UMs had the lowest tamoxifen plasma concentrations (Tamoxifen median plasma concentrations in µg/L: PM=290, IM=210, EM=190 and UM=170). Furthermore, there was a statistically significant variation of Z-tamoxifen plasma concentrations among the phenotypic groups when PMs and IMs were grouped together: Z-tamoxifen median plasma concentrations in µg/L: PM=IM=130, EM=90 and UM=62 (P=0.03).

Conclusion This is the first study in the Middle East that evaluates the frequencies of CYP2D6 polymorphisms in a Lebanese breast cancer population and the effect of these polymorphisms on tamoxifen metabolism. We have shown that CYP2D6 phenotype is significantly correlated with Z-tamoxifen levels. PMs and IM group had the highest Z-tamoxifen plasma concentrations; in contrast UMs had the lowest Z-tamoxifen plasma concentrations. Further recruitment and follow up are being performed to assess for the role of these polymorphisms in disease outcome.

P4-01-17 TIMP-1 Over-Expression Confers Resistance of MCF-7 Breast Cancer Cells to Fulvestrant.

Bjerre CA, Vinther L, Belling K, Schroih Rasmussen A-S, Li J, Lin X, Han Z, Wang J, Boland L, Jensen V, Nielsen BS, Soekilde R, Gupta R, Lademann U, Brünnen N, Stenvang J. Faculty of Life Sciences, University of Copenhagen, Copenhagen, Denmark; Technical University of Denmark, Lyngby, Denmark; Aarhus University, Aarhus, Denmark; BGI-Shenzhen, Shenzhen, China; Exiqon A/S, Vedbeak, Denmark

Background: Endocrine resistance represents a major challenge in the management of estrogen receptor (ER) positive breast cancer. Currently no predictive biomarkers for endocrine resistance in ER-positive breast cancer patients are in clinical use. In a clinical study, patients with metastatic breast cancer and high levels of serum Tissue Inhibitor of Metalloproteinases-1 (TIMP-1) had less benefit from endocrine therapy than patients with a lower level of serum TIMP-1 [1].

Therefore, we evaluated the association between TIMP-1 and response to endocrine therapy using an in vitro approach. We have previously presented initial results on TIMP-1 and response to endocrine therapy [2].

Materials and Methods: MCF-7 cells were stably transfected with pcDNA3.1(Hyg)-TIMP-1 plasmid, and a panel of 11 subclones with different expression levels of TIMP-1 was generated. TIMP-1 expression levels were confirmed using enzyme-linked immunosorbent assay (ELISA).

Four subclones with high or low TIMP-1 expression were analyzed for the growth response to estrogen, 4-hydroxytamoxifen and fulvestran. These four subclones were analyzed for protein expression by western blotting. All subclones were analyzed by whole human genome oligo microarrays 4x44K for determination of gene expression levels. Data were analyzed using the limma R/Bioconductor package. Pair-end Solexa sequencing was applied to selected subclones with high and low TIMP-1 levels to identify transcriptional changes.

Results: High expression of TIMP-1 was associated with resistance to fulvestran, whereas growth response to either estrogen or 4-hydroxytamoxifen was independent of TIMP-1 expression levels. High expression of TIMP-1 protein and mRNA was associated with undetectable levels of progesterone receptor (PgR) protein and mRNA whereas ER protein and mRNA levels were unaffected by TIMP-1. To characterize the potential role of TIMP-1 in estrogen signaling we analyzed the expression of reported estrogen-responsive genes and no general change was observed. We identified genes that correlated positively or negatively to TIMP-1 expression. Among the identified genes was PgR, which is a direct target for ER.

Conclusion: Our data suggest that a high expression of TIMP-1 in vitro is associated with resistance to fulvestran but not to 4-hydroxytamoxifen. Estrogen-regulated genes are not generally affected by changes in TIMP-1 expression levels and therefore TIMP-1 appears to affect endocrine resistance through other mechanisms than globally regulating ER signaling. However, high expression of TIMP-1 is associated with loss of PgR and this may be related to the resistance towards fulvestran.

References:


P4-01-18 AP-1 Blockade Potentiates the Anti-Tumor Effect of Endocrine Treatment and Reverts the Resistant Phenotype in Hormone Receptor-Positive Breast Cancer.

Malorni L, Giuliano M, Migliaccio I, Wang T, Creighton C, Lupien M, Hilsenbeck SG, Healy N, Mazumdar A, Trivedi MV, Jeselsohn R, Fu X, Gutierrez C, Brown M, Brown PH, Osborne CK, Schiff R. Baylor College of Medicine, Houston, TX; Hospital of Prato, Italy; The University of Texas M.D. Anderson Cancer Center, Houston, TX; Dana-Farber Cancer Institute and Harvard Medical School, Boston, MA; Dana-Farber Cancer Institute, Boston, MA; Beth Israel Deaconess Medical Center, Boston, MA; Dartmouth Medical School, Lebanon, NH; UH College of Pharmacy, Houston, TX

Background: Resistance to endocrine therapy is a major clinical issue. The transcription factor AP-1 is a key regulator of cell growth and survival as well as a downstream signaling component of several pathways deregulated in endocrine-resistant breast cancer. We have previously shown that acquired endocrine resistance is associated with increased AP-1 activity. AP-1 has also been shown to interact with and modulate the ER network and transcriptional program, especially under hyperactive growth factor signaling, which is commonly associated with endocrine resistance. We hypothesized that interfering with AP-1 function would circumvent endocrine resistance possibly due to its role in modulating ER transcriptional activity.

Methods and results: We inhibited AP-1 function by a genetic approach. We used two different MCF7 clones stably transfected...
with a Doxycycline (Dox)-inducible dominant-negative (DN) c-Jun (MCF7/Tet-Off Tam67 clones 62 and 67) and two vector-alone control MCF7 clones. Xenografts of these clones were established in ovariectomized nude mice supplemented with estrogen (E2). Mice were then randomized to continued E2 supplementation (control) or to endocrine therapy with either estrogen deprivation (ED) or tamoxifen (Tam), all in the presence or absence of Dox to induce the DN c-Jun expression. AP-1 blockade in both MCF7/Tet-Off Tam67 clones significantly enhanced sensitivity to Tam by reducing time to tumor size halving (p=0.014 and p=0.006 for clone 62 and 67, respectively) and time to complete tumor disappearance (p=0.001 and p=0.0034 for clone 62 and 67, respectively). Similar results were obtained with ED treatment. In addition, AP-1 blockade significantly delayed the onset of Tam resistance by increasing time to tumor size doubling (p=0.028). Furthermore, induction of DN c-Jun resulted in a dramatic shrinkage of growing tumors after long-term Tam treatment, suggesting reversal of endocrine resistance with AP-1 blockade. None of the above effects was observed in control clones upon Dox removal. Interestingly, no significant effect of AP-1 blockade was observed on E2-stimulated tumor growth. IHC analysis showed that AP-1 blockade induced tumor response by reducing proliferation (i.e., decreased % of Ki67- and phospho-Histone 3-positive cells) and by inducing apoptosis (i.e., increased % of cleaved caspase 3/7-positive cells). Bioinformatic analyses were conducted to intersect our MCF7 xenograft/Tam-resistant gene signature and the datasets of genes associated with ER DNA-binding sites obtained by whole-genome ER cistrome analysis under estrogen or epidermal growth factor (EGF) stimulation of MCF7 cells. A significant enrichment of the genes associated with the EGF-unique ER DNA-binding sites was observed within our Tam-resistant signature (p=2E-16). Remarkably, 90% of these DNA binding sites harbored an AP-1 motif. Conclusions: We show that AP-1 blockade increases tumor sensitivity and circumvents resistance to endocrine therapy, thus warranting the development of AP-1-targeted therapy to improve endocrine treatment outcomes. Overall, we suggest that AP-1 is critical in induction of a switch in the ER transcriptional program and may be a new hallmark of endocrine resistance.

P4-01-19
SRC-1 Expression Is a Prognostic Factor of Breast Cancer-Specific and Disease-Free Survival in Inflammatory Breast Cancer.

Arias-Pulido H, Ahmed RD, Chaher N, Vargas J, Joste N, Lomo L, Qualls C, Azziez S, Terki N, Bouzid K, Prossnitz E, Royce M. University of New Mexico, Albuquerque, NM; Centre Pierre et Marie Curie, Algiers, Algeria

Background: Steroid receptor coactivator (SRC-1 to 3) proteins drive nuclear receptor transcriptional activity by modulating transcriptional processes such as chromatin modification, transcription initiation, chain elongation, and RNA splicing. In patients who display resistance to endocrine treatment, SRC-1 has been implicated in the conversion of tamoxifen anti-agonist activity to its agonist profile. SRC-1 expression is a prognostic of DFS in breast cancer, but its role in inflammatory breast cancer (IBC) remains unknown.

Materials and Methods: SRC-1 protein levels were measured by immunohistochemistry in 103 IBC and 11 normal, non-cancer related samples, diagnosed at the Centre Pierre & Marie Curie (Algiers, Algeria). Staining results were correlated with protein levels of ER, PR, and HER2, and clinico-pathological variables, breast cancer-specific (BCSS) and disease-free survival (DFS), and response to tamoxifen treatment.

Results: SRC-1 was positive in all normal breast tissue, as well as in 66% of IBC samples. SRC-1 positivity was associated with worse OS (P<0.001) and DFS (P=0.004). SRC-1 expression correlated with worse BCSS (P=0.011), and marginally with DFS (P=0.046) in tamoxifen-treated patients, but strongly with DFS (P=0.007) in patients who received no endocrine therapy as indicated by Kaplan-Meier and log-Rank analyses. Multivariate analysis showed that SRC-1 (Hazard Ratios (HR): 5.2; 95%CI: 2.0-13.6; P<0.001) and Her2 (HR: 3.1; 95%CI: 1.2-8.1; P=0.02) expression were independent prognostic markers of BCSS. SRC-1 (HR: 4.8; 95%CI: 1.9-12.2; P<0.001), Her2 (HR: 2.4; 95%CI: 1.0-5.7; P=0.05), and hormone treatment (HR: 0.4; 95%CI: 0.2-0.8; P=0.01) were independent prognostic markers of DFS.

Discussion: Determining prognosis and predicting response to endocrine treatment is of paramount importance in the search for personalized treatment. SRC-1 expression predicts worse OS, DFS, and, similar to non-IBC, predicts DFS independently of endocrine therapy. This study highlights the importance of SRC-1 expression as a strong prognosticator of BCSS and disease recurrence in IBC patients.

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P4-01-20
Specific Anti-Proliferative Profile of Endoxifen for MCF-7 Cell Compared to 4-OH Tamoxifen.

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Background: Endoxifen and 4-hydroxy tamoxifen are both active metabolites of tamoxifen and exert anti-tumor activity for ER+ breast cancer. However, only small numbers of paper reported biological profile of endoxifen.

Methods: Cells were incubated with various concentration of endoxifen (Z)4-Hydroxy-N-desmethyl Tamoxifen (<10% with (E)-form) for 6 days and viability (anti-proliferative index) was assessed by cell counting kit-8. For seeking the influence of ERβ, two independent ERβ expressing clones of MCF-7 cells were established by stable transfection of ERβ vector to MCF-7. Western blotting was done with ERα, ERβ, PgR and HER2 antibodies.

Results: IC50s of endoxifen for MCF-7 were 100 nM in estradiol (E2) deprivation condition and 500 nM in the presence of 1nM E2. These are significantly higher than those of 4-OH tamoxifen, which are 10 nM without E2 and 50 nM with E2, respectively. From the previous reports, plasma endoxifen concentrations in breast cancer patients were ranged from 20 to 200 nM according to CYP2D6 phenotype. Anti-proliferative index of 20 nM endoxifen for MCF-7 was 0.7 (indicating 30% reduction in proliferative activity), and 200 nM endoxifen was 0.5 in the absence of E2. These were 0.95 (20 M) and 0.75 (200M), respectively, when incubated with 1 nM E2. 10 nM 4-OH tamoxifen, which is mean concentration in patient plasma, showed about 0.6 anti-proliferative index with no regards to E2 condition. Without E2, endoxifen reduced ERα expression as previously reported, however, endoxifen increased ERα amount in the presence of E2 accompanied with decrease of HER-2 expression. This alteration of ERα and HER2 expression were identical to those
observed using 4-OH tamoxifen. There was a weak tendency that ERβ expressing MCF-7 clones showed favorable response to endoxifen compared to parent MCF-7, but this was not significant.

Conclusion:
Endoxifen is active agent for MCF-7 cell, however, it requires 10-fold higher concentration compared to 4-OH tamoxifen to achieve IC 50. Difference of anti-proliferative effect between 20 nM and 200 nM of endoxifen are relatively small, and it might result in minor difference for clinical response, as expected from recent clinical studies of CYP2D6. This is the first time to show that endoxifen has opposite effect to ERα protein level depending on the presence or absence of E2. ERβ expression did not enhance activity of endoxifen in contrast to previous report.

P4-01-21
An Estrogen-Inducible Transcription Factor FOXP1 Promotes Estrogen-Dependent Cell Proliferation of Breast Cancer Cells and Is Associated with 5-Year Disease-Free Survival in Patients with Tamoxifen-Treated Breast Cancer.
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Estrogen signaling pathways are involved in the growth and development of breast tumors through the activation of estrogen receptor α (ERα). ERα is expressed in most breast cancers and involves estrogen-dependent proliferation by acting as transcription factors activating the expression of target genes. Various coregulators and transcription factors are associated with ERα-mediated transcriptional control of target genes. Therefore, a comprehensive understanding of estrogen signaling pathways in breast cancer is required for both treatment and diagnosis of the disease. Forkhead box P1 (FOXP1) is a member of the forkhead box transcription factor family and has been reported to be associated with various types of tumors. Here, we investigated the expression pattern of FOXP1 by immunohistochemistry in a series of 133 invasive breast cancers and compared it with clinicopathological factors. The expression of FOXP1 was detected in nuclei in 89 cases (67%) and correlated positively with tumor grade and hormone receptor status, including ERα and progesterone receptor (PgR), and negatively with pathological tumor size (pT). And in ERα-positive MCF-7 breast cancer cells, we demonstrated that FOXP1 mRNA was upregulated by estrogen and that ERα recruitment to ER binding sites within the FOXP1 gene region identified by ChIP-chip analysis was increased. We also demonstrated that proliferation of MCF-7 cells was increased by exogenously transfected FOXP1 and decreased by FOXP1-specific siRNA. Moreover, in MCF-7 cells, FOXP1 enhanced estrogen response element (ERE)-driven transcription. Finally, FOXP1 immunoreactivity was significantly more elevated in relapse-free breast cancer patients treated with tamoxifen than in relapse patients treated with it. Taken together, these results suggest that FOXP1 plays an important role in proliferation of breast cancer cells by modulating estrogen signaling and that FOXP1 immunoreactivity might be associated with the estrogen dependency of breast cancer clinically, which may predict favorable prognosis in the patients treated with tamoxifen.

P4-01-22
Cyclin D1 and Its Prognostic Value in Planning of Endocrine Therapy for Women of Postmenopausal Age with Breast Cancer.
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Background: Nowadays tamoxifen still remains the primary drug in breast cancer endocrine therapy. However, its application is limited due to the resistance of tumor cells. The search of adequate biomarkers is one of the most actual problems in prognosis of effectiveness of tamoxifen adjuvant therapy. The most prospective biomarker is cell cycle regulator cyclin D. Besides its central role in cell cycle regulation cyclin D1 modulates the estrogen receptors activity and can influence on the effectiveness of treatment with antiestrogens and aromatase inhibitors.

The objective of our work was to evaluate the effectiveness of tamoxifen in adjuvant therapy of hormone-receptor-positive breast cancer in women of postmenopausal age with cyclin D1 expression. Material and Methods.
To evaluate the effectiveness of tamoxifen adjuvant application in women with cyclin D1-positive T1-4N0-3M0 breast cancer we have researched 2 retrospective groups of 70 patients that have been on regular medical check-up for a period of 5 years or that had previously undergone treatment. On the basis of the archive histological material we have revealed cyclin D1 in tumor cells.

Results: The expression of cyclin was classified in 4 levels: negative, low, moderate and high. Patients with lack of cyclin D1 expression or with low qualitative value (according to our data less than 30%) have no neoplastic process progression throughout the 5 years of tamoxifen adjuvant therapy. These women were both with axillary lymph nodes affection and without it (+N; -N). On the contrary, women with moderate and high cyclin D1 expression (more than 30%) had a relapse of tumor and distant metastasis is prognosed in 5 years of observation (p=0,005). Moderate level of cyclin D1 expression was observed in 45 (64%) patients and 28 (62%) of them had progression with metastasis in bones, 9 (20%) metastasis in soft tissues, 2 (4%) metastasis in lungs and 6 (14%) relapse in postoperative scar. High expression was revealed in 25 (35%) women and 17 (68%) of them had bone affection, 7 (28%) soft tissue metastasis and 2 (4%) tumor relapse in postoperative scar. Lung metastasis were not observed. The average period of tumor relapse and progression of neoplastic process in patients with cyclin D1-positive breast cancer is 20 months.

Discussion: The results of our work suggest that women with hormone-receptor-positive cyclin D1-negative breast cancer on early stages have more prolonged non-relapse period during the tamoxifen adjuvant therapy. Patients with cyclin D1-positive breast cancer are less sensitive to tamoxifen treatment and in adjuvant regime should receive therapy with other effective equivalent drugs (aromatase inhibitors).

P4-02-01
Bis(2-Ethylhexyl) Phthalate: A Potential Endocrine Disruptor Confers Letrozole Insensitivity and Induction of Aromatase Activity in Breast Cancer Cells.
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Exposure to endocrine disrupting compounds (EDC’s) is an important determinant of mammary gland development and mammary tumor pathogenesis. The risk of breast cancer in adulthood is known to
be influenced by environmental factors experienced as a fetus. Fetal exposure to dietary factors or certain pharmaceuticals and environmental chemicals that affect or mimic steroid hormones increase predisposition to breast cancer. The mechanisms by which EDC’s alter epigenetic programming, differentiation and deregulation of ductal branching of mammary glands are poorly understood. A high ratio of estrogen receptor α (ERα) to ERβ has been recently found to be associated with poor prognosis and aggressive disease in breast cancer patients. Preliminary studies from our laboratory revealed that bis(2-ethylhexyl) phthalate (DEHP) is a possible EDC which induces cell proliferation in T47D, MCF7, MCF7/Aro breast cancer cells in vitro and confers insensitivity to letrozole, a third generation aromatase inhibitor. DEHP increased cell proliferation (MTT assay) in a dose dependent manner and was non toxic to T47D, MCF7 and MCF7 7 Aro cells. 1, 10 and 100 nM concentrations of DEHP up regulated ERα and down regulated ERβ at the mRNA level. Hypermethylation in the estrogen receptor beta (ERβ) promoter was observed after treatment with 1nM DEHP for 24 h, suggesting that DEHP alter epigenetic programming through changes in the DNA methylation to modify the chromatin structure and change the accessibility of DNA to transcription factors. When breast cancer cells were treated with letrozole (200nM) and DEHP (1nM) together, they survived, whereas those treated with letrozole alone did not survive. Aromatase mRNA and enzyme levels were higher in the DEHP treated cells by comparison with sham control MCF7/Aro cells. Nonmalignant MCF-10A breast epithelial cells showed a proliferation advantage (MTT assay) by up regulation of ERα as well as aromatase expression when treated with 1nM DEHP. Aromatase transgenic mice treated with 10μg/kg b.wt of DEHP over a period of 30 days resulted in the excessive ductal branching density and epithelial outgrowth in the mammary gland. The present data suggest that dietary accumulation of DEHP through plastic food containers, water bottles, etc. may contribute to risk of hormone sensitive breast cancer in women and chemoinosensitivity and or drug resistance in breast cancer patients.

**P4-02-02**

The Natural Fetal Estrogen Estetrol (E4), Causes Anti-Estrogenic Effects in Women with Endocrine-Responsive Positive Early Breast Cancer.

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Background: Estetrol (E4) is a natural fetal estrogen which exerts estrogenic effects on reproductive organs and on the bone, and effectively reduces menopausal symptoms. In contrast to other estrogens, however, E4 has estrogen-antagonistic effects on breast cancer cell lines in vitro and in the rat DMBA model, which would make it a suitable Hormone Replacement Therapy (HRT) in breast cancer patients, particularly in women who are being treated with aromatase inhibitors. Patients and Methods: We have investigated the effect of 14 days pre-operative treatment with 20 mg E4 per day on tumor proliferation, apoptosis, ER-receptors, PgR receptor and several endocrine parameters in a prospective, randomised, double-blind, placebo-controlled, neo-adjuvant study in 15 pre- and 14 postmenopausal women with estrogen-receptor positive early breast cancer. Results: Estetrol induced a significant increase of SHBG, a significant decrease of FSH in postmenopausal women and no increase of gonadotrophins in premenopausal women. Estetrol had no effect on Ki67 expression and on apoptosis-related Bax and Bcl-2, but the apoptosis index in tumor tissue increased significantly. Systemic IGF-1 levels decreased significantly. Surprisingly the intratumoral epithelial ER-alpha expression decreased significantly, whereas the ER-beta expression showed a trend to increase. Conclusion: Our data suggest that E4 may be suitable and safe for HRT in women with spontaneous or induced menopausal symptoms, since apoptosis increases, IGF-1 decreases and no unfavourable effects are observed on Ki67, Bax and Bcl-2. The decrease of ER-alpha and the increase of ER-beta suggest a mechanism of action, explaining why the natural fetal estrogen E4 has estrogen-antagonistic effects on breast cancer tissue.

**P4-02-03**

Biological Functions of Estrogen Receptor-beta and Its Variants in Breast Cancer.

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Background: The role of estrogen receptor alpha (ERα) in breast cancer has been studied extensively; yet, much less is known about full-length ERβ (ERβ1) and even less about its 4 variant forms (ERβ2-5). We have recently implicated a role for ERβ1 in sensitizing ERα expressing breast cancer cells to anti-estrogens. However, the ability of ERβ2-5 to modulate ERα and ERβ1 activity, and their association with cancer development, progression, and response to estrogen and anti-estrogens are not well understood. Here, we provide evidence that the presence of ERβ variants may be of diagnostic and clinical relevance for breast cancer patients and describe the development and characterization of a novel, highly specific monoclonal antibody (MC10) that is able to detect their expression in tumor biopsies. Methods: Transient transfection and luciferase assays were used to determine the transcriptional activity of ERβ2-5 in response to E2 and anti-estrogens alone or in combination with ERα and ERβ1. A novel monoclonal antibody targeting all ERβ variants (MC10) was developed and characterized. The sub-cellular localization of ERβ2-5 was determined via confocal microscopy. Finally, the MC10 antibody was used to assess ERβ positivity in breast tumors and was compared to that of another monoclonal antibody which only detects ERβ1. Results: Unlike ERβ1, ERβ2-5 do not activate an estrogen response element (ERE) in response to E2 and instead, slightly repress the activity of this reporter construct. Expression of ERβ2-5 does not significantly alter the transcriptional activity of ERβ1 following E2 treatment. However, ERβ2, 3 and 5, but not ERβ4, significantly enhance the E2-induced transcriptional activity of ERα. Interestingly, expression of ERβ3, 4 and 5, but not ERβ2, enhance the ability of anti-estrogens to block ERα mediated transcriptional activity. Confocal microscopy revealed that ERβ1 and 2 are almost exclusively localized to the cell nucleus. However, ERβ3-5 exhibit significant cytoplasmatic and peri-nuclear localization. Immunohistochemistry of breast cancer biopsies using the MC10 antibody revealed multiple staining patterns including tumors which exhibit primarily nuclear staining and others primarily cytoplasmatic, both in the presence and absence of ERα. These results are in contrast to the almost exclusive nuclear staining obtained on the same tumors with an ERβ1-specific antibody. Conclusions: ERβ variants exhibit variable sub-cellular localization patterns and can influence the function of ERα, both in response to E2 and anti-estrogens. Therefore, the differential expression of ERβ variants and their cellular localization may influence breast cancer progression and/or therapeutic responses. The use of ERβ antibodies which do not detect all ERβ variants, or the use of a single ERβ antibody which does not discriminate between ERβ1 and its variants,
is unlikely to reveal the complete biological significance of total ERβ expression in breast cancer and may in part explain the conflicting studies which have been reported for ERβ in the field.

**P4-02-04**

Androgen Receptor (AR) Expression in a Cohort of Patients (pts) with Triple Negative Breast Cancer (TNBC).


**Background:** TNBC, defined by the absence of ER, PR, and HER2, is associated with higher risk of recurrence and BC-related mortality, earlier age at diagnosis, menarche, and 1st pregnancy, increased parity, higher BMI, and African-American/Hispanic race. TNBC is a heterogeneous group. Using gene expression analysis, our group described a subset of AR+ ER/PR- BC that exhibits androgen-dependent growth. *In vitro* studies confirmed the functional role of AR and showed that growth could be abrogated by antiandrogens.(Doane et al 2006) We translated this work into a phase II trial of bicalutamide in pts with AR+ ER/PR- metastatic BC (MBC). (NCT00468715) We now describe the prevalence and clinicopathological characteristics of AR+TNBC in primary disease in a single-institution retrospective cohort.

**Methods:** We identified 1,032 pts with resectable, TNBC (ER/PR<1%; HER2<2+/FISH<2.2) who had surgery at MSKCC from 1998-2006. Exclusion criteria: neoadjuvant chemotherapy, prior radiation, inflammatory/MBC. IRB approval was obtained. We constructed tissue microarrays (TMA) from 210 primary tumors (>1 cm) with each tumor represented by three 0.6mm cores. AR was tested with DAKO antibody (Clone AR441; dilution 1:500). TMAs were digitized with a Mirax scanner. MetaMorph image analysis software was used to quantify the ratio of DAB staining to hematoxylin signal. A ratio >1 SD above mean was defined as AR+. AR+ cores were manually reviewed; false positives due to core artifact were excluded. To evaluate clinicopathological variables and differences in recurrence-free survival (RFS) and overall survival (OS) by AR status we used chi-square/t-tests and Kaplan-Meier methods/log-rank test, respectively.

**Results:** 169 pts had adequate cores for image analysis/quantification of AR. 10% of pts tested AR+ (17/169). Median (med) followup: AR++=6 years (yr), AR+=5.6yr. Demographic/clinicopathological variables: Table 1 (ages in med yr). Overall med age=54yr (29-84). Adjuvant chemotherapy received: AR+ 82%, AR- 87%, p=0.40; 77% received anthracycline/taxane-based therapy. Med time to distant metastasis (DM)=2.1yr (0.2-6.2yr). We were unable to demonstrate a difference in 5yr RFS (69% vs. 77%; p=0.37) or OS (68% vs. 84%; p=0.25) between AR+ and AR- TNBC.

**Conclusions:** Consistent with our prospective study, AR is expressed in ~10% of TNBC tumors in this retrospective cohort. The pts in our dataset may be older, postmenopausal, more likely to self-report white race and have T1-2/N0-1 BC. No statistically significant differences were observed in demographic/clinicopathological variables or survival outcomes between AR+ and AR- TNBC. Additional TMA data from our database will be presented.

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**P4-02-05**

The Regulation of Artemin Signalling by IGF-1 in Mammary Carcinoma Cells.

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Artemin is a neurotrophic signalling factor which belongs to the glial-derived neurotrophic factor (GDNF) family of ligands. Artemin acts as a survival, proliferation and migration factor for a number of neurological cell types, by signalling through the RET (rearranged during transfection) receptor and, in most cases, the GDNF receptor (GFR)-α3 co-receptor. Recently, a number of published studies have implicated Artemin as a potential oncogene in several cell types, including mammary carcinoma cells. Other studies further indicate that Artemin may influence cancer progression and tamoxifen resistance in some breast cancers. Available clinical data has demonstrated that increased Artemin expression is correlated with decreased overall patient survival in breast cancer patients and a poor outcome in tamoxifen treated breast cancer patients.

Here we investigate interaction between the Artemin and the insulin-like growth factor-1 (IGF-1) signal transduction pathways. Using mammary carcinoma cell lines, we demonstrate that IGF-1 treatment increases the endogenous expression of both Artemin and its endogenous receptors, RET and GFRα3. Semi-quantitative RT-PCR assays demonstrated that IGF-1 stimulated mRNA expression of Artemin as well as RET and GFRα3 in wild-type MCF-7 and ZR-75-1 cells in a time-dependent and dose-dependent manner. The same effect was not observed in wild-type T47D cells where IGF-1 did not increase Artemin mRNA expression.
We also demonstrated that forced expression of Artemin in MCF-7 cells consistently enhanced the response of these cells to IGF-1 in a number of cell function assays. Forced expression of Artemin significantly enhanced IGF-1-mediated stimulation of total cell number in MCF-7 cells. Consistent with this, Artemin enhanced IGF-1-mediated stimulation of S-phase entry and cell survival. In a soft agar assay, forced expression of Artemin also enhanced IGF-1-mediated stimulation of colony formation. Conversely, depletion of Artemin expression using siRNA abrogated the response to IGF-1 stimulation in MCF-7 cells. Artemin depletion significantly decreased IGF-1-stimulated increase in total cell number by decreasing IGF-1-stimulated cell proliferation and protection from apoptotic cell death. In addition, forced expression of Artemin in MCF-7 cells reduced cell sensitivity to the IGF-1 receptor small molecule inhibitor, AG1024. In conclusion, we have demonstrated that IGF-1 increases Artemin mRNA and protein expression in the breast cancer cell lines MCF-7 and ZR-75-1 and have identified potential cross-talk between the Artemin and IGF-1 signalling pathways in MCF-7 cells.

P4-02-06

Progestrone Receptor Expression Predicts Poor Outcome in Estrogen Receptor Positive, Lymph Node Negative Breast Cancer – A Population Based Study.

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Background: Estrogen receptor (ER) positive, lymph node (LN) negative breast cancer usually carries a good prognosis; endocrine therapy is often the only adjuvant treatment. However, a small proportion of such patients do badly (possibly representing those with luminal B cancers identified by gene expression profiling). A clinically applicable method for identifying this subgroup of poor prognosis ER+, LN- patients is required to offer them more intensive adjuvant therapy.

Material and methods: A consecutive, unselected series of 1072 new, operable breast cancer patients diagnosed between 2000 and 2004 was studied for ER and progestrone receptor (PR) expression, HER2 status and standard pathological and demographic parameters. ER and PR expression were scored on immunohistochemistry (IHC) on preoperative, diagnostic core biopsies using the “Quickscore” method. This ensured optimal fixation and tissue processing avoiding the variable fixation of resection specimens and the well-described sampling error of tissue micro-array (TMA) methodologies. PR expression is frequently heterogeneous resulting in false negative scores in at least 12% of cases by TMA analysis. HER2 status was assessed using IHC with dual color FISH for cases scoring 2+.

Adjuvant therapy was prescribed using standard protocols; all patients with ER+ disease received adjuvant endocrine therapy. Follow up data were obtained from the oncology database, and the registrar of deaths for the date and cause of death. All deaths not attributable to breast cancer were censored at the date of death. Accordingly, the primary end-point was breast cancer-specific overall survival. Survival analysis was carried out by Kaplan-Meier survival curves analysed by the Log-Rank test. Multivariate analysis was carried out using Cox’s regression.

Results: Overall, PR- cancers had a worse prognosis than PR+ (p<10^-12, Hazard Ratio 3.40), even in the ER+ (p=0.006, HR 1.86), LN- (p=10^-4, HR=5.33) and LN+ (p=10^-11, HR=3.26) sub-groups. In the ER+ LN- group, the absence of PR expression predicted worse prognosis (88% vs 96% 8 year survival, p=0.0003) with a hazard ratio of 3.75. This is considerably more significant than Ki67 IHC scoring reported for a similar group of patients in other studies (HR 2.22). Multivariate analysis demonstrated that PR expression was an independent prognostic variable second only to LN status and more powerful than ER.

Discussion: Patients with ER+, LN-, PR- breast cancers have a significantly worse prognosis than those with ER+, LN-, PR+ cancers. Unlike Ki67, PR IHC uses a simple, cost effective, standard methodology (as for ER) and should identify patients who may require chemotherapy in addition to endocrine therapy in this group of otherwise good prognosis patients.
tumors have a significantly deteriorated prognosis and seem to be a patient group, which should be investigated concerning drug resistance mechanisms.

**P4-02-08**

**Obesity-Induced Aromatase Expression in the Breast Microenvironment Promotes Estrogen Receptor Activity Independent of Circulating Estradiol Levels.**

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Epidemiological studies indicate that obesity increases the risk of postmenopausal breast cancer by approximately 50%. In the past, researchers have hypothesized that elevated estrogen synthesis by the peripheral adipose tissue may be the principal mediator of breast tumorigenesis in this population, which primarily develops estrogen receptor alpha (ERα) positive breast cancer. However, obesity is also accompanied by an elevation in growth factor and cytokine signaling, and these pathways have been linked to tumorigenesis. In addition, certain growth factor and cytokine family members can promote aromatase expression in both the epithelial and stromal tumor compartments. Consequently, we hypothesized that obesity increases the risk of postmenopausal breast cancer via elevated aromatase expression and/or activity in the local mammary tissue.

To test our hypothesis, we investigated how ERα activity in mammary epithelial cells was influenced by adipose stromal cells (ASC) cultured under obesity-associated conditions, including high cell density and exposure to elevated levels of circulating growth factors and cytokines. For the latter condition, sera was obtained from postmenopausal women, pooled by BMI category (lean: 18.5-24.9; obese: ≥30), and applied to the ASC, which were originally derived from women undergoing reduction mammoplasty. High ASC density was achieved via the protocol previously published by Dr. Li. Preliminary data indicated that both elevated cell density and sera from obese postmenopausal women induces greater aromatase expression in ASC, indicating that multiple factors may be contributing to the increased local aromatase expression seen with obesity. We are currently exploring the signaling pathways responsible for obesity’s upregulation of ASC aromatase expression and will present these results at the meeting. Intriguingly, exposure to conditioned media from both the high density and obese sera-exposed ASC enhanced ERα activity in MCF-7 mammary epithelial cells, independent from exogenous estradiol but dependent on the presence of androgens, suggesting an important role for the aromatase enzyme in this observation. To expand on this finding, we plan to investigate the effect of ASC conditioned media on different markers of cancer aggression, including proliferation and survival, and assess the degree to which these effects depend on estradiol. Through further examination of obesity’s impact on signaling pathways in both the epithelial and stromal tumor compartments, we ultimately hope to identify more effective chemopreventive and therapeutic regimens for the high-risk obese postmenopausal population.

**P4-02-09**

**Estrogen-Related Receptor α Mediates Insulin-Like Growth Factor-1 Dependent Migration in Breast Cancer Cells.**

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The orphan nuclear estrogen-related receptors (ERR) have homology to steroid hormone receptors. Unlike classical steroid hormone receptors, ERs do not have known ligands but are transcriptionally activated upon binding to nuclear receptor coregulators such as PGC1α, PGC1β, and PPRC1. ERRs regulate energy metabolism and are accordingly expressed in tissues that require high energy production. ERRs recognize their own ERRE (estrogen-related receptor response element) gene promoters, but can also activate transcription viaERE (estrogen receptor response element) promoters, representing the ability of ERRs to cross participate in ERα regulatory pathways in the absence of estrogen. Of the three family members (α,β,γ), ERRα is the most highly expressed in breast tissue. ERRα expression was found to be increased in ERα negative breast cancers and associated with poor prognoses. ERRα and its coactivators can increase the expression of a critical mediator of breast tumor angiogenesis and metastasis, VEGFA. We have previously shown that VEGFA is regulated by insulin and IGF receptor activation and hypothesized that ERRα may be a mediator of this finding. We therefore explored whether ERRα and its coactivators are necessary for IGF-1/insulin dependent regulation of VEGFA and cell migration. REDa was expressed in breast cancer cell lines, but no correlation was observed with breast cancer subtypes. Reducing endogenous ERRα levels by shRNA reduced IGF-1-dependent migration in MDA-MB-231 cells. These results were confirmed using an ERRα specific inverse agonist (XCT-790), which also inhibited MDA-MB-231 cell migration. Modulating ERRα mRNA levels via shRNA resulted in decreased VEGFA mRNA levels in MCF7L cells and increased VEGFA mRNA levels in MDA-MB-231 cells, while the inverse agonist XCT-790 caused an increase in VEGFA mRNA levels in both cell lines. Reducing ERRα levels did not affect the expression of the IGF1R, IRS, IRS-1, IRS-2, PI-3K-Akt-mTOR dependent signaling pathway. Neither ERRα protein nor mRNA levels were modulated by IGF-I or insulin. We conclude that ERRα is important for mediating IGF-1 dependent migration, but have shown that ERRα is not directly regulated by the IGF signaling. We will next explore the role of ERRα’s coregulators (PGC1α, PGC1β, and PPRC1) in the regulation of VEGFA by the IGF/insulin pathway given their central role in regulating ERRα’s function.

**P4-02-10**

**The Relationship between Estrogen Receptor Gene Polymorphism and Mammographic Density in Postmenopausal Women.**

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**Background**

Assess the relation between the presence of PVUII and XBAI polymorphisms in the estrogen receptor alpha gene and mammographic density in postmenopausal women.

**Methods**

For the present analysis, 189 postmenopausal women who had never used hormonal therapy and who did not have clinical or mammographic features were selected. Based on the ACR-BIRADS 2003 classification, the mammographic density was determined by three independent readers (two subjective ratings and one computerized – Adobe Photoshop 7.0 software). Blood samples were available to extract DNA according to KIT GFX â protocol. PCR-RFLP (Polymerase Chain Reaction – Restriction Fragment Length Polymorphism) was then used to identify the polymorphisms. PCR-RFLP (Polymerase Chain Reaction – Restriction Fragment Length Polymorphism) was then used to identify the polymorphisms.

**Results**

There was a high degree of agreement among the three readers for mediating IGF-1 dependent migration, but have shown that ERRα is not directly regulated by the IGF signaling. We will next explore the role of ERRα’s coregulators (PGC1α, PGC1β, and PPRC1) in the regulation of VEGFA by the IGF/insulin pathway given their central role in regulating ERRα’s function.
be classified in dense breasts group (p=0.003) and the presence of both wild alleles was associated with fibro glandular tissue replacement by fat (p=0.02).

Conclusions There was no significant association of the PVUII polymorphism in the estrogen receptor alpha gene with mammographic density (p=0.34). However, the XBAI polymorphism was observed at a higher mutated homozygous frequency in women with dense breasts and there was an increased frequency of wild-type homozygous and heterozygous women with fat-replaced breasts (p=0.01).

P4-02-11
Insulin-Like Growth Factor I Promotes Estrogen Receptor Positive Breast Cancer Cell Proliferation, in Part, through CYP1A1 Signaling.
Rodriguez M, Becker M, Yee D, Potter D. University of Minnesota, Minneapolis, MN

Activation of the transmembrane tyrosine kinase insulin-like growth factor 1 receptor (IGF-1R) contributes to breast cancer progression. Nonetheless, the mechanisms by which the IGF-1R contributes to breast cancer progression need to be better characterized to further develop therapeutic strategies that target this pathway. It is known that cytochrome P450 monoxygenases synthesize metabolites from endogenous and exogenous sources that promote breast cancer proliferation. Here we propose that activity of the IGF-1R modulates the activity of CYP1A1 to promote breast cancer cell proliferation. We have found that IGF-1R activation by treatment with IGF1 (5nM) for 4hrs induces cytochrome P450 1A1 (CYP1A1) mRNA levels in estrogen receptor (ER) positive line T47D-CO, but not ER negative line MDA-MB-231. Treatment with IGF1 promotes the proliferation of ER positive lines T47D-CO and MCF7. Interestingly, we found that treatment with IGF1 only partially compensates the growth-inhibition induced by CYP1A1 knock down by siRNA, suggesting that IGF1 requires CYP1A1 for full promotion of cell growth. These preliminary data suggest that IGF-1 signaling functions, in part, through CYP1A1 to promote ER positive breast cancer cell proliferation. The mechanism of IGF-1 signaling through CYP1A1 is novel and may represent an effector mechanism for IGF-1. Further understanding of the role of CYP1A1 downstream of the IGF-1R may allow novel approaches for inhibition of breast cancer cell proliferation downstream of the IGF-1R.

P4-02-12
Plasma Estradiol Levels and Degree of Estrogen Receptor Positivity by Image Analysis in a Large Cohort of Breast Cancer Cases: Results from the Nurses’ Health Study.
Collins LC, Frieling GW, Ahern TP, Hu R, Hankinson SE, Tamim RM, Beth Israel Deaconess Medical Center, Boston; Harvard Medical School, Boston; Channing Laboratory, Brigham and Women’s Hospital, Boston; Harvard School of Public Health, Boston

Background: Breast cancer risk is strongly associated with endogenous reproductive hormones. There is also good evidence to suggest that endogenous estradiol levels are more strongly associated with the development of estrogen receptor (ER) positive breast cancers than with ER negative cancers. However, the degree of ER positivity in those cancers that develop is not yet established. Therefore, we sought to evaluate the proportion and intensity of tumor cells demonstrating ER positivity using image analysis in a large cohort of breast cancers for which we also had plasma estradiol levels.

Design: Tissue microarray (TMA) sections of breast cancers from women enrolled in the Nurses’ Health Study were immunostained for ER (1D5, Dako Corporation, Carpinteria, CA). Slides were then scanned using the ScanScope slide scanning system (Aperio Technologies Inc., Vista, CA) and ER staining was assessed for both intensity and percent of nuclei staining positively using a specific algorithm, Nuclear v9, which evaluates nuclear size, intensity, roundness, curvature, compactness, and elongation. Plasma samples were collected prior to the date of diagnosis for all women in the study and estradiol levels were determined by radioimmunoassay following extraction and celite column chromatography.

Results: Image analysis data for ER was available for 691 breast cancers. The mean percent of tumor cell positivity was approximately uniformly distributed, with a mean of 44.5%, (median=41.3%, max=99.6%). The proportion of tumor cells staining for ER from the TMA cores was positively associated with patient age (p=0.0001), and postmenopausal status (p=0.008), while inversely associated with tumor grade (p=0.0001). Among women with image analysis data for ER, plasma estradiol data were also available for 320 postmenopausal women. Estrogen receptor positivity (by proportion or intensity) did not vary according to plasma estradiol levels (p=0.17).

Conclusion: While high concentrations of plasma estradiol are more strongly associated with the development of estrogen receptor positive breast cancer versus estrogen receptor negative cancers, the degree of estrogen receptor positivity in the breast cancers that develop is not influenced by this variable.

P4-03-01
Stat5 regulates the P13-Kinase/Akt1 pathway during mammary gland development and promotes neoplastic transformation in a mouse model for Cowden syndrome.
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Background: The Jak/Stat pathway responds to extracellular signals (i.e. cytokines) to promote physiological responses at the transcriptional level. In the developing mammary gland, Jak2/Stat5 activity has also been shown to cause mammary tumors in a number of transgenic models and we have previously shown it plays a crucial role in the early neoplastic events of prolactin and ErbB2-induced tumorigenesis. However, the mechanisms and target genes by which Stat5 promotes cell survival and neoplastic transformation remain to be elucidated and are the focus of this current study.

Materials and Methods: To assess the functions of Stat5 during mammary development, gain-of-function Stat5 was expressed in a number of transgenic models and we have previously shown it plays a crucial role in the early neoplastic events of prolactin and ErbB2-induced tumorigenesis. However, the mechanisms and target genes by which Stat5 promotes cell survival and neoplastic transformation remain to be elucidated and are the focus of this current study.

Materials and Methods: To assess the functions of Stat5 during mammary development, gain-of-function Stat5 was expressed in a spatial and temporal manner within the mammary epithelial cells of double transgenic females (Wap-TATet-Ostat5SS710F model) during lactation and involution in a doxycycline (dox)-inducible manner. Mammary tissues were collected for histological examination along with mRNA, protein, and chromatin immunoprecipitation (ChIP) analyses. HC11 mouse mammary epithelial cells were infected to overexpress wildtype or gain-of-function Stat5, a constitutively active prolactin receptor, or combinations of both. Finally, the functional cooperation between Stat5 and P13-kinase/Akt signaling in promoting mammary cancer was assessed by expressing hyperactive Stat5.
specifically within the mammary epithelium of females harboring a knock-in PtenG129E mutation found in patients with Cowden Syndrome (PtenG129E MMTV-TATA TetO-Stat5S710F model).

Results: Dox-induced expression of active Stat5 during involution overrides pro-apoptotic signaling and sustains p-Akt1 levels along with expression of the PI3-kinase and Akt1, which are normally downregulated at the transcriptional and protein levels during involution. ChIP analysis revealed that Stat5 binds two consensus sites in the Akt1 promoter during lactation whereas this is lost by the second day of involution. Furthermore, Stat5 promotes the transcriptional activation of Akt1 through a novel mammary-specific promoter and this suggests a mechanism for Stat5 in cell survival both in mammary development and breast cancer. Preliminary results also suggest that forced expression of active Stat5 in the mammary glands of PtenG129E females accelerates the development of mammary intraepithelial neoplasia (MIN) lesions predisposed by the loss of PI3K/Akt inhibition.

Discussion: In this study we demonstrate that Stat5 regulates PI3-kinase/Akt1 signaling during mammary gland development and this provides a mechanism for enhancing cell survival during lactation and for allowing apoptosis through loss of this regulation during involution. These findings further suggest that Jak2/Stat5 signaling may play an important role in the development of breast cancers induced through aberrant PI3-kinase/Akt signaling (i.e. Cowden Syndrome) and that targeting Jak2/Stat5 activity might be a suitable strategy for prophylyactic intervention.

P4-03-02
High Fat Diet-Induced Postmenopausal Obesity Promotes Tumor Angiogenesis and Breast Cancer Progression in Age- Relevant Ovariectomized Mice.
Gu J-W, Young E, Patterson SG, Makey KL, Huang M, Tucker KB, Chinchar E, Miele L. University of Mississippi Medical Center, Jackson, MS

Background: Obese postmenopausal women have 50% higher risk of breast cancer than non-obese women. The mechanisms of postmenopausal obesity-induced breast cancer are poorly understood due to lack of the established animal model that mimics postmenopausal obesity related to breast cancer progression.

Material and Methods: Using age-relevant C57BL/6 mice, this study determined whether postmenopausal obesity increases VEGF expression, tumor angiogenesis, and breast tumor growth. Ovariectomy (OVX) was performed in 12 sixty week-old female mice (life span is about 140 weeks), then followed by a low-fat (5%, LF, n=6) or a high-fat (60%, HF, n=6) diet for 12 weeks. In the 8th wk of the dietary program, 10^6 E0771 (mouse breast cancer) cells were injected in the left fourth mammary gland. Tumor size was monitored using dial calipers for 4 wk. Body weights were monitored weekly. At the end of the experiment, blood samples, visceral fat, and tumors were collected for measuring VEGF expression using ELISA and intratumoral microvessel density (IMD) using CD31 immunochemistry.

Results: Body weight was significantly increased in OVX/HF mice, compared to OVX/LF group (55.3±1.7 vs. 41.5±1.5 g; P<0.01). There was a two-fold increase in the ratio of visceral fat/BW in OVX/HF mice, compared to those in OVX/LF group (0.062±0.005 vs. 0.032±0.003; P<0.01). Postmenopausal obesity significantly increased breast tumor weight over the control (4.6±2.63 vs. 1.98±0.27 g; P<0.01) and IMD (173±3.7 vs. 139±4.3 IM#/mm²; P<0.01). Tumor VEGF levels were higher in OVX/HF mice, compared to OVX/LF group (73.3±3.8 vs. 49.5±4.3 pg/mg protein; P<0.01). Plasma VEGF levels (69±7.1 vs. 48±3.5 pg/ml) and visceral fat VEGF levels (424.4±39.5 vs. 208.5±22.4 pg/mg protein) were significantly increased in OVX/HF mice, compared to OVX/LF group, respectively (n=6; P<0.01). Interestingly, adipose tissue primary culture showed that subcutaneous fat released more VEGF, compared to visceral fat (6.7±3.14 vs. 0.9±0.16 pg/mg tissue; n=6; P<0.01). The abdominal subcutaneous fat expressed more VEGF proteins than visceral fat in OVX/HF mice (692±72 vs. 431±44 pg/mg protein; n=6; P<0.01). There was a strong positive linear correlation between increased breast tumor weight and visceral fat weight in OVX mice (R²=0.7379; N=12; P<0.01). However, there was no significant difference in heart, or kidney weight/body weight ratio between postmenopausal obesity (OVX/HF) mice and the control mice (OVX/LF).

Discussion: Our observations indicate that ovariectomy plus a high fat diet with the inoculation of E0771 (mouse ER+ breast cancer) cells in female wild type >60 week old mice can mimic human obesity-induced postmenopausal breast cancer. The increased tumor angiogenesis in postmenopausal obese mice was correlated with increased breast tumor growth, adipose tissue mass, and adipokines such as VEGF. These findings support the hypothesis that postmenopausal obesity promotes tumor angiogenesis and breast cancer progression, possibly through increased adipose tissue mass and adipokines such as VEGF that could systemically and locally affect breast cancer progression.

P4-03-03
Therapeutic Sensitivities of Mouse Models of Human Breast Cancer.
Usary JE, Darr DB, Zhao W, Balletta LD, Aparicio AP, Karginova OA, Jordan JL, Perou CM. University of North Carolina, Chapel Hill, NC

Genetically engineered mouse (GEM) models have provided a wealth of information regarding the genetic causes of cancer, but their utility for preclinical drug evaluation has not been well examined. Here we have used three mammary tumor GEM models that represent three human breast cancer subtypes and have evaluated their sensitivities to chemotherapy and to three biologically targeted agents. We have selected three mouse models that resemble human breast cancer subtypes based upon common gene expression profiles; Basal-like tumors are represented by the C3(1)-T-antigen (C3-TAg) model, Luminal B tumors are represented by the MMTV-Neu model, and the P53 null transplant T11 line represents the newly described Claudin-low breast tumor subtype. On each of these models we have tested the therapeutic efficacy of: four chemotherapeutics (doxorubicin, carboplatin, paclitaxel, and cyclophosphamide), two chemotherapy combinations (carboplatin/paclitaxel and doxorubicin/cyclophosphamide), and three biologically targeted agents (erlotinib, lapatinib, and ABT-888, alone and combined with selected chemotherapy). The results from individual models were as follows: The MMTV-Neu tumors were sensitive to the single-agent chemotherapeutics carboplatin and cyclophosphamide, and cyclophosphamide dramatically increased overall survival of the MMTV-Neu mice. The targeted agents lapatinib and erlotinib were extremely effective; lapatinib produced a near complete regression in every MMTV-Neu mouse tested and both compounds lead to greatly increased survival times. In the Claudin-low T11 line, the tumors were very sensitive to cyclophosphamide. Alone and in combination with doxorubicin, cyclophosphamide was the only chemotherapeutic able to successfully cause tumor regression in this model. None of the biological inhibitors were effective as single agents in these mice, nor were they effective
in combination with chemotherapeutics other than cyclophosphamide. In the C3-Tag basal-like model, carboplatin alone and in combination with other drugs caused volume reduction in some of the tumors. Erlotinib was able to cause volume reductions in a third of the treated C3-Tag tumors, which revealed a heterogeneity of response within this GEM strain. None of the single agent treatments significantly increased overall survival in these mice. Those combination treatments that were effective showed a range of responses from tumor regression to slowed progression.

Finally, we closely examined the heterogeneous responses of the C3-Tag tumors to carboplatin/paclitaxel and performed expression profiling of sensitive and resistant tumors. We identified a gene signature from these treated mouse tumors that was able to predict pathological complete response of human patients receiving multagent taxane-containing neoadjuvant chemotherapy regimens. These results show that genomically selected GEM models can recapitulate findings seen in human tumors (like lapatinib responsiveness in HER2+ tumors and carboplatin sensitivity in basal-like tumors) and that GEM models can potentially be used to develop biomarkers and to test new drug combinations prior to their being tested in humans.

P4-03-04
Identification of Molecular Targets for Cancer-Initiating Cells Using a Triple-Negative Breast Cancer Mouse Model.
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Background: Triple-negative breast cancer (TNBC), which is negative for estrogen receptor, progesterone receptor, and HER2, cannot be targeted with hormonal or anti-HER2 agents and frequently relapses. Thus, innovative molecular targeted therapies for TNBC are needed. Cancer-initiating cells (CICs) play a crucial role in tumor recurrences. TNBC has a much higher proportion of CICs compared with the other breast cancer subtypes, reflecting TNBC’s clinical aggressiveness. However, qualitative or molecular traits of TNBC CICs have not been fully elucidated. We established a TNBC mouse model that successfully recapitulated human-like TNBC in vivo (SABCs 2010 abstract P1-03-02). We here report the phenotypes and molecular features of CICs in our TNBC mouse model. Methods: We created this animal model by transferring Hras(L146V) into Ink4a/Arf-knockout (KO) mouse mammary epithelial cells (MECs) in vitro and inducing tumors in mammary fat pads of recipient mice. The induced TNBCs were digested, and single suspended cells were reacted with antibodies to candidate CIC markers CD29, CD24, CD44, Sca1, CD61, and CD49f. Expression patterns of the tumor cells were analyzed by FACS. The induced TNBCs were CICs in our model. Genome-wide expression patterns for CD49f dividing cells than that of CD49f dividing cells. We thus determined that CD49f cells were CICs in our model. Genome-wide expression patterns for CD49f cells and CD49f cells were compared by paired t-test, and over- and underexpressed genes in CD49f cells were functionally annotated by Ingenuity pathway analysis. The top-ranked bioprocess of overexpressed genes in CD49f cells was hepatic satellite cell activation (HSMA), followed by pantothenal and Coenzyme A biosynthesis, and HER2 signaling in breast cancer. HSMA is known to result in liver fibrosis due to collagen deposits in response to liver damage. In CD49f cells in our model, collagen family proteins and the upstream endothelin-A receptor were overexpressed and significantly correlated with HSMA. Conclusion: We identified CICs in a mouse TNBC model and revealed their molecular profile. We plan to analyze expression of HSMA-associated proteins in human TNBC. Our first step is to determine whether endothelin-A receptor is a therapeutic target in human TNBC.

P4-03-05
Development of an Inducible Estrogen Receptor Co-Activator PELP1 Mammary Tumor Model.
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Despite treatment advances, breast cancer remains the second most lethal malignant disease for women worldwide. Given the importance of estrogen receptor (ER) and hormone-dependent nature of breast cancer, pharmacologic agents were developed to either modulate ER functions or reduce circulating estrogens levels. Although targeted endocrine therapies significantly reduce mortality in patients with estrogen sensitive (ER+) tumors, both de novo and acquired resistance limits efficacy. A critical need for identifying more precise diagnostic/prognostic biomarkers and novel therapeutic targets prompted deeper investigation into ER-coregulatory protein function and regulation. ER-coactivator PELP1 mediates both nuclear and extra-nuclear estrogen signaling and crosstalk with growth factors. PELP1 is deregulated in hormone-driven cancers, associates with undifferentiated invasive breast adenocarcinomas and an independent prognostic biomarker in assessing clinical outcome of luminal-like breast cancer patients. Collectively, several studies suggest PELP1 is an ERζ co regulator with tumorigenic potential. However, the in vivo significance of PELP1 deregulation during initiation and progression of breast cancer is unknown. To determine the role of PELP1 overexpression in mammary tumorigenesis, we generated an inducible transgenic murine model. Transgene construct (pTetOPELP1) consists of a full-length human PELP1 cDNA linked to luciferase gene reporter through an internal ribosomal entry site (IRES). PELP1 transgene was purified and microinjected into mouse zygotes to generate pTetOPELP1 mice. Founder mice were identified by Southern blot analysis, of genomic DNA extracted from tail biopsies, for transgene integration through germline transmission. pTetOPELP1 mice were bred with mammary gland-specific rTA mice (MMTVrTA) to produce MMTVrTA-TetOPELP1 hitransgenic mice. Potential founder mice were identified by polymerase chain reaction and breed to establish two independent transgenic lines. Transgene expression was induced in adult female hitransgenic animals with 200mg/ mL of doxycycline administered in drinking water. Concurrent expression and activity of the luciferase gene reporter was detected specifically in the mammary gland by in vivo bioluminescence imaging, luciferase assay and RT-PCR. Mammary epithelial-specific expression of PELP1 was validated by immunohistochemistry.
and Western blot analysis. PELP1-mediated morphological and histological changes were analyzed by examining carmine-stained whole mounts and H&E-stained mammary glands sections. Our early findings with MMTVrtTA-TetOPELP1 bitransgenic mice (n=30) early preneoplastic changes and hyperplasia were evident as early as 12 weeks and the formation of mammary tumors by 8 months of age following PELP1 induction by doxycycline treatment. By utilizing the tetracycline-regulatory system, we created a novel, inducible and mammary gland-specific PELP1-expressing transgenic model for future in vivo studies into molecular mechanisms of PELP1-mediated mammary tumorigenesis.

P4-04-01
Identification of a Unique Mammary Cell Type Expressing Mesenchymal Markers, but Capable of Multilineage Epithelial Differentiation.
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We have identified a previously uncharacterized cell population within the epithelial compartment of the post-pubertal mouse mammary gland. These cells can be found as singlets or as like pairs in both the cap and body cell layer of terminal end buds (TEB) of developing ducts, and are ultimately localized in a suprabasal position distributed throughout the mature mammary ductal tree. In differentiated ducts, these cells typically extend long processes that contact multiple surrounding luminal epithelial and myoepithelial cells suggesting a function in cell-cell communication. In younger animals, identified cells do not express epithelial markers including the major cytokeratins (CK) 5, 6, 8, and 14, or E-cadherin, nor do they express myoepithelial cell markers such as smooth muscle actin or p63. However, in 20 week old non-parous females, these cells can give rise to epithelial cell types expressing CK 5, 6, 8, or 14. In transplantation assays, epithelial fragment and whole mammary gland transplantation did not indicate a major role in regenerative growth of mammary ducts. Transplantation of dissociated epithelial cells and wound-healing assays are in progress. Taken together, these observations suggest that this unique cell population possess stem cell-like features in that they are capable of self-renewal, and of giving rise to all major differentiated cell types within the mouse mammary gland.

P4-04-02
Smoothened Function as a G-Protein Coupled Receptor in Mammary Epithelial Cells.
Villanueva H, Vishal AP, Birnbauer L, Plummer NW, Lewis MT. Baylor College of Medicine, Houston, TX; National Institute of Environmental Health Sciences, Research Triangle Park, NC
Background: Hedgehog signaling orchestrates many key developmental processes, including cell fate determination and tissue patterning in the embryo, as well as tissue homeostasis and stem cell maintenance in the adult. Aberrant hedgehog signaling has been implicated in several cancers including skin, brain, prostate and breast. Previous work in our laboratory demonstrated that overexpression of activated Smoothened (SMO), a key effector in hedgehog signaling, leads to mammary hyperplasia, and is overexpressed in DCIS and IBCs.

Hypothesis: Smoothened promotes mammary gland proliferation by functioning as a G-protein coupled receptor(GPCR) and couples to one or more members of the PTX-sensitive Gα, family of heterotrimeric G-protein subunits.

Experimental Design and Methods: Expression of Gα1, subunit(s) was tested by qRT-PCR & immunofluorescence microscopy of mammary epithelial cells (MECs) and whole mammary glands from SMO and WT mice. We analyzed mammary glands from single Gα1, subunit null mice for developmental abnormalities. Genetic studies are underway in which SMO mice are crossed to Gα1, 2, and 3 null mice.

P4-03-06
Development and Comparative Characterization of Metastasis in Newly Developed Pre-Clinical Models of Inflammatory Breast Cancer.
Chu K, Mu Z, Alpaugh KR, Fernandez S, Freiter EM, Wu H, Zook MB, Barsky SH, Cristofanilli M, Robertson FM. The University of Texas MD Anderson Cancer Center, Houston, TX; Fox Chase Cancer Center, Philadelphia, PA; University of Nevada School of Medicine, Reno, NV; Nevada Cancer Institute, Las Vegas, NV
Background: Inflammatory breast cancer (IBC) is the most metastatic variant of breast cancer. It is associated with a poor survival rate (40% 5-year survival) despite appropriate multidisciplinary care. For such an aggressive type of cancer, IBC has been understudied, in part due to the lack of adequate numbers of cell lines and mouse models that recapitulate the human disease. To expand our understanding of IBC, we have obtained all of the previously developed and characterized IBC cell lines and models including Mary-X, SUM149, SUM190, KPL-4, MDA-IBC-3 and have developed two new IBC models, designated as FC-IBC01 and FC-IBC02, using tumor cells derived from pleural effusion of IBC patients.

Materials and Methods: Each of these IBC cell lines has been luciferase (LUC)-tagged, allowing the growth of orthotopic injection or subcutaneous implantation to be evaluated by bioluminescent imaging (BLI). Alternatively, the LUC-tagged IBC cells can be injected via either intra-cardiac or intravenous route of delivery, which promotes rapid tumor colonization, resulting in both visceral and skeletal metastasis. Growth of IBC tumors can then be monitored immediately using BLI, thus eliminating the lag time needed for the physical detection of palpable tumors. BLI imaging also allows for monitoring of the kinetics and location of development of metastatic lesions. Whole transcriptome analysis was performed on IBC cell lines and xenograft tissues to define the heterogeneity of IBC as a distinct variant of breast cancer.

Results: These models have allowed us to identify micro-metastatic foci in multiple sites distant from the IBC primary tumor in each of these models of IBC and allow the quantitation of anti-tumor and anti-metastatic effects of targeted therapeutics as single agents and as the potential synergy of combinations of agents. As an example, injection of LUC-tagged IBC cell lines such as SUM149-Luc, into the left ventricle of NOD.Cg-Prkdc<sup>scid</sup> Il2rg<sup>tm1Wjl</sup>/SzJ mice allows the metabolic tumor burden to be monitored longitudinally by whole animal BLI, which can be validated at necropsy and by immunohistochemical analysis. Whole transcriptome analysis of pre-clinical models of IBC reflect the molecular subtypes observed in IBC patients, with the majority of IBC models being of the basal like, luminal B and Her2 amplified. Discussion: First time analysis of known and newly developed pre-clinical models of IBC allows a more complete analysis of IBC as a distinct variant of breast cancer. Furthermore, these approaches allow rapid evaluation of the promising targeted therapeutics identified based on whole transcriptome analysis of both IBC patient tumors and pre-clinical models developed from IBC patients. We believe that this extensive collection of LUC-tagged IBC cell lines is an invaluable tool for IBC research since the cell lines encompass the broad spectrum of IBC heterogeneity.
To elucidate downstream signaling events triggered by SMO activation, we are treating SMO derived MECs cultured in a 3D matrix and whole gland organ cultures with inhibitors of Gαi effector molecules and assessing proliferation status. We will also employ GTPγS binding assays using primary MECs from SMO overexpressing mice to test whether SMO can activate Gαi proteins in mammary gland epithelium.

**Results and Discussion:** Treatment of MMTV-Cre dependent SMO hyperplasias with pertussis toxin (PTX), a potent inhibitor of Gαi family of G-protein signaling, significantly attenuates mammary gland hyperproliferation. This result supports the hypothesis that Smoothened functions as a GPCR interacting with one or more members of the Gαi family.

We have now found that Gαs and Gαi3 null mice display an increased number of terminal end buds and a more completely filled fat pad relative to WT control littermates. Our data suggest that SMO-expressing cells induce proliferation of neighboring WT cells and that low-level proliferation in SMO-αi3 null mice should provide evidence that these two proteins interact in the developing mammary gland. When associated with biochemical data, these studies ought to provide functional insight into the mechanism behind SMO-driven mammary hyperplasias.

This work offers potential clinical implications towards breast cancer treatment as there exist therapeutic agents targeting the hedgehog signaling pathway in clinical trials that were developed by solely treating SMO derived MECs cultured in a 3D endocytic machinary and can have a huge impact on the cellular and coordinately elevated in ER+ve breast cancers. Specifically in the luminal B subtypes, they are significantly associated with a markedly worsened outcome. In vitro, in MCF7 cell line, we observe an increased EGFR stability and MAPK signaling in Rab25 overexpressing stable clones which could be reversed with shRNA knockdown of Rab25. Loss of either Rab25 or RCP inhibited survival and growth of MCF7 lines under serum free conditions suggesting that Rab25 can potentially facilitate growth factor independence in cancer cells. Most importantly, we report that Rab25 enhances mitochondrial ATP production in breast and ovarian cancer cells and significantly reduces acidification of the ECM. Our ongoing experiments are directed towards understanding the mechanistic link between Rab25 mediated effects on EGFR/pERK signaling and its effects on cellular metabolism.

**P4-05-02 Early Developmental Exposures to a High Carbohydrate/High Fat Diet Affect Glucose Metabolism and Mammary Cancer Susceptibility.**

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**Background:** The obesity epidemic in the U.S. has substantially increased the number of people with altered glucose metabolism. Alterations in metabolic programming, resulting from early exposures can affect development, metabolism as well as propensity to later diseases, including cancer. The Warburg effect describes a mechanism by which diet-induced hyperglycemia and hyperinsulinemia can contribute to cellular transformation. To investigate the effect of a hyperinsulinemia-, hyperglycemia-inducing (HI/HG) diet on metabolic programming and susceptibility to mammary cancer, we exposed developing mouse pups during three developmental stages: gestation, lactation and post-weaning.

**Materials and Methods:** Female SENCAR mice were fed either a mildly restricted, defined, “chow like” control diet (DR) or a HI/HG, high sucrose/high fat (HS/HF) diet. At 14 weeks, both DR and HS/HF fed female mice were bred and the resulting offspring were randomized into 8 groups to model all combinations of gestational, lactational and post-weaning dietary exposures. Body weights (BW) were recorded weekly and Glucose Tolerance Tests (GTTs) were conducted on the female offspring at 10-12 weeks of age. Starting at 7-9 weeks of age, mice received 20 μg/day of DMBA or vehicle by daily gavage to induce mammary carcinogenesis.

**Results:** Animals in the DR/DR/DR (gestational diet/nursing diet/post-weaning diet) and HS/DR/DR groups had the lowest average BW and retained normal response to glucose, while mice in the DR/HS/HS and HS/HS/HS groups had the highest average BW. Interestingly, animals born to DR-fed and nursed by HS/HF mothers and weaned...
onto a HS/HF diet (DR/HS/HS) were the most glucose intolerant. In response to DMBA, animals in the different dietary regimens partitioned into high, moderate or low mammary tumor incidence groups. Mice fed consistent diets throughout gestation, lactation and post-weaning (DR/DR/DR or HS/HS/HS) had intermediate tumor incidence. However, mice exposed to DR during gestation and/or lactation and then switched to a HS/HS diet at weaning had the highest tumor incidence and shortest latency. Mice exposed to a HS/HF diet during gestation and/or lactation and then switched to a DR diet at weaning had the fewest mammary tumors and longest latency.

**Conclusion:** Our data indicate that substantial changes in the type and abundance of calories during gestation and/or lactation had long-lasting impacts on BW, glucose metabolism, and mammary tumorigenesis. Since BW did not consistently correlate with GTT results, the effects of dietary manipulation on obesity and glucose metabolism appear to be distinguishable. Results also indicate that diet-induced changes in the glucose metabolism of the mother profoundly affected tumor susceptibility in the exposed female offspring. These findings support the contention that rising levels of obesity, metabolic syndrome, and diabetes, especially in young individuals, may contribute to the increasing incidence of early onset breast cancer.

**P4-05-03**

**Mechanism Underlying Metabolic Heterogeneity in Breast Cancer Subtypes.**

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**Background:** Several distinct subtypes of breast cancer (Luminal, Basal-like and Her-2+) have been identified by gene expression profiling of breast cancers and cell lines. Although much is known about the regulation of cell signaling in each breast cancer subtype, little is known about subtype-specific energy metabolism and its regulation. The majority of cancers, including breast cancer, acquire an accelerated metabolic index as part of the transformation process. One of the most studied metabolic changes in cancer, referred to as the Warburg effect, is the increased uptake of glucose and its conversion to lactate; which is released from the cell, thus creating an astringent tumor microenvironment with high lactate and low pH. Accumulation of lactate in the tumor microenvironment presents cancer cells with a potential rich carbon source that could be exploited when the preferred nutrient sources, glucose and glutamine, are not abundant or available. Thus uptake and conversion of lactate to pyruvate and then entry into the TCA cycle and oxidative phosphorylation could generate energy that could potentially allow cancer cells to survive until other nutrients become available or until the cancer cells can invade and migrate toward nutrient rich environments, in other words, until they spread locally and metastasize.

**Methods and Results:** Examination of 59 breast cancer cell lines shows differences in expression of numerous proteins and enzymes involved in cellular metabolism, including the lactate transporters (MCT), the enzyme lactate dehydrogenase (LDH), and glucose transporter proteins (GLUT). We found that MCT1 expression is lost in the Luminal subtype and correlates with loss of LDHB and the regulator and chaperone of MCT1, CD147 (Basigin). Conversely, MCT1 is highly expressed in Basal-like and normal cell lines, along with CD147 and LDHB. While monocarboxylate transporter proteins can transport bi-directionally, MCT1 preferentially transports lactate into the cell, while MCT4 transports lactate out of the cell. The loss of MCT1 in luminal cells suggests a distinct energy metabolism in this subtype versus basal-like cells. The basal-like subtype has been further categorized into Basal and Claudin-low subtypes. Claudin-low cells express stem-like markers such as CD44+/CD24-, EMT markers such as vimentin, and are low expressers of the Claudin proteins. We found that MCT1 and CD147 are highly expressed and differentially regulated in Claudin-low cell lines as compared to normal cells. We have evidence that high lactate (10 mM) as a sole energy source delays ATP loss and apoptosis in MCT1 expressing cells, but not in luminal cell lines that lack MCT1. Thus, claudin-low tumors may benefit from local lactate production providing an unexpected energy source.

**P4-05-04**

**Arachidonic Acid-Induced Elevated Expression of 5-Lipoxygenase Is Linked to Metastatic Migration of Breast Cancer Cells.**

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**Background:** In invasive breast cancers, cancerous cells spread outside the ducts of the breast and metastasize to lung and other tissues. Although the rapid arachidonic acid (AA) metabolism and concomitant increase of eicosanoid molecules are involved in the proliferation and invasion of breast cancer cells, the exact mechanism by which AA metabolites regulate these phenomena are not well understood. Here, we show that leukotriene B4 (LTB4), one of the metabolites of AA, which is produced by the action of 5-lipoxygenase (5-LOX), causes the proliferation and metastatic migration of breast cancer cells.

**Material and Methods:** MDA-MB-231 (invasive breast cancer cells) and MCF7 (non-invasive breast cancer cells) were used in this study. Briefly, cells were treated with AA (100 μM) and nordihydroguaiaretic acid (NDGA, 10 μM), and the secreted eicosanoids were characterized by HPLC. Immunofluorescence microscopy was performed to elucidate the expression and intracellular localization of 5-LOX. The metastatic migration was analyzed by wound-healing assays.

**Results:** Our results suggest that while MDA-MB-231 cells produce high levels of PGE2 and PGD2, MCF7 cells synthesize excess HETE compounds (HETE5 and HETE8). Interestingly, MDA-MB-231 cells, when stimulated with AA, show the increased syntheses of LTB4 (~3 fold) and decreased PGE2 and PGD2 (~2 fold). In MCF7, on the contrary, AA treatment reduced the syntheses of all eicosanoids. Furthermore, the expression of 5-LOX in MDA-MB-231 cells was also increased by ~2 fold. We observed that AA promotes cell migration in MDA-MB-231 cells, which could be blocked by NDGA, a generic inhibitor of LOX enzymes. Because LTB4 production and its binding with BLT receptors are linked to IL-8 secretion, we measured the level of IL-8 synthesized by both MCF7 and MDA-MB-231 cells. The results suggest that MDA-MB-231 cells secrete excess IL-8 (~2 fold) when stimulated with AA, suggesting that LTB4 and IL-8 interactive pathways are important for cancer metastasis.

**Discussion:** Our results demonstrate that LTB4 synthesis is linked to the metastatic migration of MDA-MB-231 cells, and that it therefore should be considered as a target for developing new drugs for the treatment of invasive breast cancers.
P4-05-05
Imaging Mass Spectrometry Based Lipid Metabolites Analysis for Breast Cancer.
Ide Y, Nishio T, Hosokawa Y, Matsunuma R, Koizumi K, Ogura H, Shiiya N, Setou M. Hamamatsu University School of Medicine, Hamamatsu, Japan

Background:
Activation of lipid metabolism is an early event of carcinogenesis and a central hallmark of many cancers including breast cancer. Recent findings argue that stearoyl CoA desaturase-1 (SCD1), a key regulator of the fatty acid (FA) composition and the endoplasmic reticulum resident enzyme that converts saturated FA (SFA) into monounsaturated FA (MUFA) is a novel regulator of carcinogenesis. The distinctive lipids composition of membrane in cancer cells and the biological functions of SCD1, however, still remain uncertain. Imaging mass spectrometry (IMS) is a mass spectrometry-based analyzing technique that enables visualization of the individual molecules without requiring antibodies. It allows comprehensive detection of a wide range of biomolecules, such as lipids. We attempted to visualize the localization of lipids in breast cancer by IMS for better understanding of cancer proliferation.

Materials and methods:
13 specimens were obtained from the primary breast cancer patients. All were Japanese woman and aged 41-86 years (mean 61.5y.o.). Only one patient received preoperative systematic therapy. 6 were estrogen receptor (ER) and/or progesterone receptor (PgR) positive and human epidermal growth factor receptor 2 (HER2) negative, 2 were ER and/or PgR positive and HER2 positive, 2 were both ER and PgR negative and HER2 positive and 2 were triple negative. IMS:
Samples were immediately chilled in liquid Hexan and stored at -80°. All specimens were sliced into 10 μm thin sections, mounted onto one indium-tin oxide-coated glass slides (Bruker Daltonics) and then sprayed by 2,5-Dihydroxybenzoic acid. Matrix assisted laser desorption ionization (MALDI) technique was used as a soft ionization method. We used time of flight (TOF)/TOF type instrument (Ultraflex, Bruker Daltonics) and all the spectrum were acquired automatically using Fleximaging software (Bruker Daltonics). Each spectral intensity at any mass-to-charge ratio (m/z) was measured at 16 regions of interest (ROI); 13 ROI were picked up from cancerous parts and 3 were from non-cancerous parts. Spectral intensities were compared and statistical analysis was performed by Mann Whitney test. The software was also used to create two-dimensional ion-density maps.

Results:
In the cancerous parts of all the 13 specimens, two distinct peaks of the molecular ions were detected at m/z 798.5 and 810.5, which were not found in the non-cancerous parts. Median intensity of the molecular ions at m/z 798.5 and 810.5 were 38.9 and 3.18 in the cancerous part, while they were 0.84 and 1.02 in the non-cancerous part (p=0.010 and 0.015, respectively). Tandem mass spectrometry analysis for these two molecules revealed that they were two kinds of phosphatidylcholine (PC), PC (16:0/18:1) and PC (18:0/18:1). Localization of the individual PC was visualized by means of IMS, which showed that in cancerous part accumulation of PCs containing MUFA was more pronounced than those containing SFA only.

Conclusions:
Two kinds of PC containing MUFA were found to highly accumulate in cancerous parts, which may suggest involvement of SCD1 in the membrane composition regulation and cancer proliferation. Further studies may thus be warranted to explore the relation between PC localization and the SCD1 expression.

P4-06-01
Withdrawn by Author

P4-06-02
Microscopic Disease in Blood and Bone Marrow Predicts Survival in Early Stage Breast Cancer.
Lucci A, Krishnamurthy S, Lodhi A, Bhattacharyya A, Hall C, Anderson A, Bedrosian I, Singh B, Kuerer H. University of Texas MD Anderson Cancer Center, Houston, TX

Background: Disseminated tumor cells (DTCs) in the bone marrow have been identified in 30% of stage I-III breast cancer (BC) patients and predict survival. Circulating tumor cells (CTCs) in the blood predict outcome in metastatic BC, but their prognostic significance in primary BC is unclear. This study determined whether: 1) DTCs and CTCs could be identified in significant numbers of non-metastatic BC patients and 2) if these cells predict relapse-free (RFS) and overall survival (OS).

Methods: Clinical stage I-III BC patients seen at a single tertiary cancer center provided informed consent to participate in an IRB-approved study involving collection of blood (7.5 ml x 2 tubes) and bone marrow (10 ml from bilateral iliac crests), and at the time of surgery for their primary BC. DTCs were assessed using an anticytokeratin antibody cocktail (MNF 116, CK 8,18, and 19, CAM 5.2, and AE1/AE3) following ficoll enrichment and cytoospin. A positive result for DTCs was defined by presence of one or more CK-positive cells meeting morphologic criteria for malignancy. CTCs were detected using the Cell SearchTM system. A positive result was defined as the presence of one or more cells per 7.5 ml blood since a threshold for positivity has not been established in non-metastatic BC. Statistical analyses used chi-square and Fischer’s exact test.

Results: We prospectively evaluated 313 patients. Mean age was 53 years, and median follow-up was 32 months. Forty-two percent of patients (131) had T1 tumors, 36% (112) T2, 10% (30) T3, and 13% (40) had T4 disease. Forty-five percent of patients (141/312) had positive lymph nodes. DTCs were identified in 29% (91/313) and CTCs in 25% (79/313) of all patients. Seven percent (21/313) of patients had both DTCs and CTCs. In the overall cohort, 26% (83/313) patients relapsed and 15% (48/313) died. Ten percent (9/91) of DTC positive patients died compared to 3% (6/222) of those who did not have DTCs (p=0.01). Similarly, 6% (7/79) of those who had CTCs died compared to 3% (8/234) of those who did not (p=0.03). Fifteen percent (12/79) of CTC positive patients relapsed compared to 6% (14/234, P=0.01) of those who were CTC negative. Simultaneous presence of DTCs and CTCs in 25% (79/313) of all patients. Seventy percent (91/313) of patients had both DTCs and CTCs. In the overall cohort, T2 tumors had 15% (40) had T4 disease. Forty-five percent of patients (141/312) had T1 tumors, 36% (112) T2, 10% (30) T3, and 13% (40) had T4 disease. Forty-five percent of patients (141/312) had positive lymph nodes. DTCs were identified in 29% (91/313) and CTCs in 25% (79/313) of all patients. Seven percent (21/313) of patients had both DTCs and CTCs. In the overall cohort, 26% (83/313) patients relapsed and 15% (48/313) died. Ten percent (9/91) of DTC positive patients died compared to 3% (6/222) of those who did not have DTCs (p=0.01). Similarly, 6% (7/79) of those who had CTCs died compared to 3% (8/234) of those who did not (p=0.03). Fifteen percent (12/79) of CTC positive patients relapsed compared to 6% (14/234, P=0.01) of those who were CTC negative. Simultaneous presence of DTCs and CTCs was a strong predictor of RFS (log rank p=0.030, HR= 2.8, 95% C.I. 1.20- 8.10) as well as OS (log rank p=0.026, HR= 3.66, 95% C.I. 1.03- 13.00) at 2 years. Combined presence of DTCs and CTCs was a predictor of outcome and these findings persisted after adjusting for variables including hormone receptor status, HER2 status, primary tumor size, grade, and preoperative lymph node status. There was no significant correlation between DTCs and/or CTCs with other primary tumor characteristics.

Conclusions: Circulating and disseminated tumor cells can be identified in a significant number of non-metastatic breast cancer patients. Both CTCs and DTCs predicted outcome, and their combined presence was an independent predictor of survival.
**P4-06-03**  
Multiplex Gene Expression of Disseminated Tumor Cells in the Bone Marrow of Breast Cancer Patients Identifies Novel Therapeutic Targets.  
Afni R, Mudalagiriyappa C, Pillai S, Fleming T, Watson M. Washington University, St. Louis, MO  
**Background:** Disseminated tumor cells (DTCs) are detected in the bone marrow (BM) of up to 40% of breast cancer patients at the time of diagnosis and are an independent prognostic factor for recurrent disease. Present techniques for detection of DTC are often laborious, and insensitive due to the molecular heterogeneity of the DTCs. We have previously optimized and validated a novel, multiplexed gene expression technology platform, Nanostring nCounter™ (NC) which counts single molecules of RNA, for the multi-marker detection of DTCs in BM at a sensitivity of 1 cancer cell per 1 million nucleated BM cells. We now validate a 36 gene panel for the detection and molecular characterization of DTCs in BM.  
**Methods:** Hybridization probes for 36 genes whose expression are associated with breast cancer, metastasis, and/or the cancer stem cell phenotype, and which exhibit no or low expression levels in normal bone marrow by qRT-PCR were developed for the NC assay. Total RNA was isolated from whole BM collected from the right and left iliac crest from breast cancer patients and healthy volunteers. 5 ug of RNA was analyzed, in duplicate with the NC assay. BM was scored positive for expression of an individual gene if expression in duplicate samples was 2 standard deviations above mean expression in a set of 11 independent normal BM samples.  
**Results:** Bilateral BM samples were analyzed prior to any therapy from 20 patients: 8 developed metastatic disease within 2-48 months (mean of 23 months) after diagnosis, and 12 had no evidence of metastatic disease with 3-5 years follow-up. Overall, expression of at least one gene in the 36-gene multi-marker panel was detected in 17 patients (85%). There was excellent correlation between individual gene expression in both the right and left iliac crest samples from the same patient. Six of the 8 patients (75%) who developed metastatic disease had detectable expression of 1-3 genes. Two genes were commonly associated with metastatic disease development. 50% (3 of 6) of the patients who had detectable expression of EBB2 in their BM developed metastatic disease, although this did not correlate with expression in the corresponding primary tumor from the same patient. 80% (4 of 5) of the patients who expressed the hedgehog pathway gene, Ptch1, in their BM developed metastatic disease.  
**Conclusions:** Our data demonstrate the feasibility of using a 36-plex NC assay to detect gene expression associated with BM DTCs in breast cancer patients. We found expression of 2 targetable genes associated with the development of metastatic disease, ERBB2 and Ptch1. ERBB2 expression in BM did not correlate with expression in the primary tumor. The molecular diversity of gene expression observed underscores the need for a multiplexed gene expression panel. Ongoing studies are evaluating the clinical utility of this assay to detect DTCs relative to existing techniques, for predicting relapse-free survival, molecular classification, and selecting appropriate targeted therapeutics based on BM DTC profiles in breast cancer patients.

**P4-06-04**  
Detection of HER2 Gene Amplification in Circulating Tumor Cells and Disseminated Tumor Cells by Fluorescence In Situ Hybridization Using OncoCEE™.  
Krishnamurthy S, Bischoff FZ, Mayer JA, Wong K, Pham T, Kuerer HM, Lodhi AK, Bhattacharyya A, Hall CS, Lucchi A. University of Texas M.D. Anderson Cancer Center, Houston, TX; Biocept Laboratories, San Diego, CA  
**BACKGROUND:** The status of HER2 gene amplification in circulating tumor cells (CTCs) and disseminated tumor cells (DTCs) might provide useful information for monitoring response to trastuzumab therapy, and may provide a basis for consideration of trastuzumab in patients with HER2 negative primary tumors who have HER2 positive CTCs and/or DTCs. The majority of techniques utilized for detection of minimal residual disease are limited in their ability to allow detailed phenotypic and genotypic evaluation of the cells. We report the utility of a microfluidic platform (OncoCEE™, Biocept, San Diego) for detecting HER2 gene amplification in CTCs and DTCs in patients with non-metastatic breast cancer.  
**METHODS:** Peripheral blood (10ml) and bone marrow (BM) (1-2ml) were collected from patients with clinical stage I-III breast cancer in acid citrate dextrose solution (BD, Franklin Lakes, NJ) and anti-clumping reagent (OncoCEE-Sure™). Mononuclear cells were recovered using a Percoll density gradient method, incubated with a mixture of 10 primary capture antibodies (Abs), introduced into CEE™ microchannels, stained with fluorescent anti cytokeratin (CK) and CD45 abs and finally processed for fluorescence in situ hybridization (FISH) using probes specific to centromere 17 (spectrum green) and HER2 (spectrum range). The ratio of HER2:CEP17 >2.2 in any CK+/CD45- and CK-/CD45- cell was regarded as positive for HER2 gene amplification.  
**RESULTS:** Peripheral blood and/or BM from 78 patients (65 BM; 70 blood; 57 matched blood and BM) with T1NO(39), T1N1 (8), T2N0(12), T2N1 (2), T2N2 (1), T2N3 (3), T3N0 (2), T3N1 (2), T3N2 (1), T4N0(5), T4N1 (3), with HER2+ (n=12) and HER2- (n=58) primary invasive breast tumors were studied. The 12 patients with HER2+ primary tumors had HER2+ DTCs in 3/12 (25%) and HER2+ CTCs in 1/9 (11%) cases respectively. HER2+ DTCs and HER2+ CTCs occurred in 12/55 (24%) and in 4/63 (6%) of the patients with HER2- primary breast tumors. HER2+ CTCs and DTCs occurred simultaneously in only 2 patients and in either blood (3) or BM (13) in the remaining patients.  
**CONCLUSION:**  
1. The cell enrichment and extraction microfluidic technology (OncoCEE™) provides a sensitive platform for evaluation of HER2 gene amplification of CTCs and DTCs.  
2. HER2+ primary tumors were associated with either HER2+ CTCs or DTCs in 25% of the patients.  
3. HER2+ CTCs or DTCs occurred in 28% of patients with HER2- primary tumor.  
4. Discordant HER2 status was contributed mainly by HER2+ DTCs occurring in HER2- primary tumors.  
5. The clinical significance of evaluating the status of HER2 gene amplification in CTCs and DTCs in the management of patients with breast cancer needs to be evaluated prospectively in larger clinical trials.
P4-06-05
Prognostic Impact of Disseminated and Circulating Tumor Cells in Patients Treated for Locally Advanced Breast Cancer.
Mathiesen RR, Nesland JM, Renolen A, Lokkveik E, Anker G, Østensen B, Lundgren S, Riisberg T, Myaaland I, Kvalheim G, Lønning PE, Naume B. Oslo University Hospital The Radium Hospital, Oslo, Norway; Oslo University Hospital, Oslo, Norway; University of Bergen, Bergen, Norway; Haukeland University Hospital, Bergen, Norway; St. Olav University Hospital, Trondheim, Norway; Norwegian University of Science and Technology, Trondheim, Norway; University Hospital of Northern Norway and Institute of Clinical Medicine, University of Tromsø, Tromsø, Norway; Stavanger University Hospital, Stavanger, Norway; Oslo University Hospital, The Radium Hospital, Oslo, Norway; University of Oslo, Oslo, Norway

INTRODUCTION:
Neoadjuvant systemic therapy (NST) in breast cancer patients is an established approach to reduce tumor size prior to surgery and to assess the clinical effect of therapy on the breast cancer disease. The current study was designed to identify primary tumor resistance factors to epirubicin and paclitaxel therapy in patients with locally advanced breast cancer (Chrisanthar et al PLoSOne 2008 and 2011). As a sub-study, the incidence of disseminated tumor cells (DTCs) and circulating tumor cells (CTCs) before and after therapy, was investigated. The aim was to evaluate the prognostic impact of DTCs and CTCs as well as to evaluate the effect of NST on DTCs and CTCs.

PATIENTS AND METHODS:
Patients with locally advanced non-inflammatory breast cancer (T3-4 and/or N2) were included in the study. The patients were randomly allocated to primary treatment either with epirubicin 90mg/m² or paclitaxel 200mg/m², with cross-over design if no response/progression, followed by mastectomy and axillary dissection. Bone marrow (BM) aspiration and peripheral blood (PB) samples were collected before NST (BM1/PB1)(n=230), at the time of surgery (BM2/PB2)(n=69; logistic reasons caused reduced sampling) and 12 months after randomization (BM3/PB3)(n=162). Detection of DTCs/CTCs was performed by standard immunocytochemical analysis of 2x10⁶ mononuclear cells stained for cytokeratin by AE1AE3 antibodies. Patient outcomes were evaluated over a 10-year follow-up period. Univariate and multivariable proportional hazards models were estimated to assess the prognostic significance of DTC for disease-free survival (DFS) and overall survival (OS).

RESULTS:
Before NST (BM1) 21.3% were DTC positive, compared to 15.9% and 26.5% at BM2 and BM3, respectively. Of those that both had BM1 and BM3 performed, 68% concordance and 22% overlap among positive cases was observed. Presence of DTCs in BM3 predicted reduced DFS (HR 2.2; 95% CI 1.3-3.7; p=0.007) and OS (HR 3.0; 95% CI 1.8-5.2; p=0.001). DTC status before NST had no impact on outcome. No difference in the results was observed after exclusion of patients with limited M1 status at diagnosis (25 and 13 of those analysed for BM1 and BM3, respectively). The incidence of CTCs before NST was 4.9% compared to 1.4% and 4.3% at PB2 and PB3, respectively. Presence of CTC before NST was associated with reduced overall survival (HR 2.4; 95% CI 1.2-5.0; p=0.018), but CTC status was not significant for DFS or at other time points. In the multivariable analysis, DTC status at BM3 remained as a prognostic factor for both DFS (HR 2.0; 95% CI 1.1-3.6) and OS (HR 2.1; 95% CI 1.04-4.2).

CONCLUSION:
In patients with locally advanced breast cancer, the presence of CTCs at the time of diagnosis identified high risk patients. However, the sensitivity of the performed CTC analysis was too low for further interpretation. Presence of DTCs 12 months after neoadjuvant therapy increased the risk for relapse and death. The best clinical utility of DTC analysis appears to be as a monitoring tool during follow up, in a “window of opportunity” for selection of patients to secondary adjuvant treatment intervention within clinical trials.

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P4-06-06
Morphological Categories of ICC-Detected CK+ Cells in Bone Marrow Have Different Prognostic Impact in Breast Cancer.
Borgen E, Synnestvedt M, Schirmer CB, Schlichting E, Nesland JM, Naume B. The Radium Hospital, Oslo University Hospital, Oslo, Norway; The Radium Hospital, Oslo University Hospital, Oslo, Norway; Ullevål Hospital, Oslo University Hospital, Oslo, Norway; University of Oslo, Oslo, Norway

Introduction: Morphological evaluation increases the specificity of immunocytochemical analysis (ICC) of disseminated tumor cells (DTC) in bone marrow (BM). In the Oslo1 cohort of primary breast cancer (BC) patients (pts), ICC-positive cells detected in BM were prospectively classified into 4 morphological groups: Tumor cells (TC) and un-interpretable cells (UIC, i.e. degenerated/fragmented cells), both significantly associated with reduced survival; and hematopoietic cells (HC) and questionable HC (Q-HC), not affecting outcome (Borgen 1999, Naume 2004). The present study revisits these categories, comparing their significance according to therapy and tumor subtype.

Materials and methods: Mononuclear cells (MNC) from BM collected at surgery of 761 pts were analyzed by standard ICC, including 2x10⁶ BM MNC stained for cytokeratin by AE1AE3 monoclonal antibodies, and corresponding isotype specific negative controls (neg co; 2x10⁶ BM MNC for most). Cells were classified as TC, UIC, Q-HC or HC. Primary tumor analyses included ptPn stage, grade, hormone receptor (HR) and HER2. Patients were followed for median 99 months. Survival analyses were performed by Kaplan Meyer and Cox regression analysis.

Results: Frequency of TC in specific test (AE1AE3) was 16.9%, UIC 14.0%, Q-HC 23.4% and HC 27.2%. By analyzing the entire cohort and numerically correcting for similar cells in neg co, only cells classified as TC or UIC predicted systemic relapse. Analysis of no adjuvant treated (NO-ADJ; n=390) and adjuvant treated (ADJ; n=361) pts separately, revealed that in NO-ADJ pts only those harboring Q-HC experienced reduced survival (DDFS: HR 2.2, 95% CI 1.2-4.1, p=0.01; BCSS: HR 2.7, 95% CI 1.3-5.5, p=0.006). In ADJ pts, only TC and UIC predicted relapse (HR 1.8, 95% CI 1.2-2.9, p=0.004) and death (HR 2.0, 95% CI 1.3-3.1, p=0.003). In multivariable analysis for NO-ADJ pts, presence of Q-HC by AE1AE3 remained significant (DDFS: HR 2.1, 95% CI 1.1-4.1, p=0.031; BCSS: HR 2.4, 95% CI 1.1-5.1, p=0.024). By subgroup analysis according to HR and HER2 (where available), prognostic impact of TC and UIC were observed only in HR+/HER2- subgroup (n=538; DDFS:HR 1.9, 95% CI 1.2-3.2, p=0.006; BCSS:HR 3.3, 95% CI 1.9-5.6, p=0.001), and not in HER2+(n=80), or triple negative pts (p=0.17). In contrast, presence of Q-HC was associated with worse outcome in triple negative pts (n=115; DDFS:HR 2.9, 95% CI 1.4-6.1, p=0.005; BCSS:HR 2.4, 95% CI 1.1-5.2, p=0.026), with no significant prognostic impact in the other two pts groups. Interestingly, pts harbouring ≥ 3 HC in AE1AE3 and in neg co together (8.5% of pts), had improved DDFS (HR 0.60, 95% CI 0.41-0.88, p=0.008) and BCSS (HR 0.57, CI 0.36-0.91, p=0.018) compared to those with fewer or no HC (p=0.029). Only 4.7% of pts with ≥ 3 HC experienced metastasis, versus 20.8% of the other.
Conclusion: Morphological DTC subgroups may differ in clinical significance according to biology/primary tumor subtype and therapy status. This emphasizes the importance of separate analyses of DTC categories and further DTC characterization. False positive cells, probably in the B-cell lineage, might signify an immune-related favorable clinical outcome.

P4-07-01
Circulating Tumor Cells, Disease Recurrence and Survival in Newly Diagnosed Breast Cancer.
Franken B, de Groot MR, Terstappen LWMM, Mastboom WJB, Van der Palen J, Tibbe AGJ. Medisch Spectrum Twente, Enschede, Overijssel, Netherlands; University of Twente, Enschede, Overijssel, Netherlands

Background: The presence of circulating tumor cells (CTC) is an independent prognostic factor for progression-free survival and overall survival (OS) for patients with metastatic breast cancer beginning a new line of systemic therapy. The current study was undertaken to explore whether the presence of CTC at the time of diagnosis was associated with recurrence free survival (RFS) and breast cancer related death.

Materials and Methods: In a prospective single center study, CTC were enumerated with the CellSearch system in 4 aliquots of 7.5 ml of peripheral blood of 504 patients before undergoing surgery for breast cancer between September 2003 and January 2009. Four hundred four had stage I-III disease and were followed for 6-90 months (median 48). One hundred had a benign tumor and served as a control group. Results: In the control group 15 (15%) had ≥1 CTC / 30 mL of blood. In stage I-III patients 76 (19%) had ≥1 CTC in 30 mL of blood of whom 16 (21.1%) developed a recurrence. In 328 patients with 0 CTC 38 (11.6%) developed a recurrence. Four year RFS was 88.4% for favorable CTC and 78.9% for unfavorable CTC (p =0.038). Breast cancer related death was 4.3% (14 of 328 patients) for favorable and 14.5% (11 of 76 patients) for unfavorable CTC (p = 0.001). CTC, PR receptor and stage were independent predictors of breast cancer related death in multivariate analysis. The corrected odds ratio after multivariate analysis for breast cancer related death in patients with CTC versus patients without CTC was 3.47.

Discussion: Presence of CTC in breast cancer patients before undergoing surgery with curative intent is associated with an increased risk for recurrence and breast cancer related death. This supports the notion that the presence of CTC is also important in the primary disease setting. In this study the sensitivity of the CellSearch assay was increased by a fourfold increase in blood volume and a threshold for unfavorable of ≥1 CTC in 30 mL of blood, which lead to a relatively high background in the control group. Thus an increase in specificity is desired before initiation of prospective multicenter trials as well as a more practical way to process larger blood volumes.

P4-07-02
Detection, Enrichment, Characterization and Propagation of Circulating Tumour Cells from Patients with Advanced Metastatic Breast Cancer.
MihalciouC, Lian J, Bertos N, Omeroglu A, Sebag M, DiBattista J, Li J, Chuangtai N, Park M, Kremer R. Royal Victoria Hospital/McGill University, Montreal, QC, Canada; McGill University, Montreal, QC, Canada

Background: Circulating tumour cells (CTCs) have attracted much attention lately due to their potential utility in diagnostic, therapeutic and prognostic applications. Characterization of these cells may indeed permit more targeted and individualized therapeutic approaches, as well as provide a means to monitor treatment response. Although detection of CTCs in peripheral blood (PB) is relatively easy using current methodologies, characterization of the CTC pool has proven more challenging due to their low abundance. Furthermore, in-vitro expansion of this elusive cell pool in mammospheres cultures has not yet been reported. In order to achieve a more complete characterization of CTCs and attempt to obtain live cells in sufficient quantity for in vitro expansion, we have used aphaeresis as a means to collect a large initial cell fraction from which to enrich CTCs from peripheral blood.

Methods: A cohort (n=17) of late stage breast cancer patients were first screened using 10ml PB. Peripheral blood mononuclear cells (PBMCs) were isolated using a Ficoll gradient and then enriched for CTCs by anti-CD45 negative selection using an automated system (RoboSep) based on magnetic bead separation. CTCs were detected by immunocytochemistry (ICC) for cytokeratin expression and patients classified as CTC-positive were selected for the aphaeresis procedure. Following collection of aphaeresis material (APM), PBMCs were isolated using a Ficoll gradient, enriched by automated anti-CD45 negative selection and characterized for a variety of markers by ICC, immunofluorescence and flow cytometry. CTCs were then cultured in serum-free medium as monolayers and suspensions. Recovery at the various steps of the isolation process was determined using PB from healthy subjects spiked with MCF-7 breast cancer cells and processed using the same approach.

Results: Recovery of spiked MCF-7 cells was about 40% after Ficoll and magnetic bead separation. Using this negative selection procedure 17/17 (100%) of subjects tested were positive at screening. Most patients (14/17) underwent aphaeresis and a large number (9,600 to 108,000) of enriched CTCs was recovered from APM in all patients tested, either as individual cells or as CTC clusters. Using dual immunofluorescence labelling, co-localization of the epithelial cell marker CK8 and the chemokine receptor CXCX4 was observed within CTCs. Furthermore, over 20% of CTCs were positive for both CK8 and ALDH1, indicative of a stem-like phenotype. APM-derived CTCs from all patients (14/14) could be propagated, both as attached cells and in suspension culture. They formed colonies in monolayer culture, and clusters in mammosphere culture, indicating stem cell-like properties. They replicated for at least three passages in mammosphere culture.

Conclusion: Our CTC detection and enrichment method using negative selection offers a distinct advantage over current methodologies, including collection of clusters, and the ability to grow and expand CTCs in serum-free culture conditions. Furthermore, these cells demonstrate breast cancer stem cell-like characteristics, the ability to replicate for multiple passages as mammospheres in suspensions and a metastatic signature.

P4-07-03
Identification of Triple-Negative Primary Breast Cancer Xenograft Models with High Numbers of Circulating and Disseminated Tumor Cells.
Giuliano M, Christy PI, Zhang X, Mao S, Contreras A, Lewis MI, Rimawi MF, Osborne CK, Schiff R, Trivedi MV. Baylor College of Medicine, Houston, TX; UH College of Pharmacy, Houston, TX

Background: Primary breast cancer xenografts, in which tumors are grown directly from patients and which maintain their original genotype and phenotype, have the potential to facilitate the study of tumor biology and progression. These models can also be instrumental in the discovery of novel therapeutic targets especially for the triple-negative (ER-, PR- and HER2-negative, TN) breast cancer.
TN breast cancer is associated with high numbers of circulating and disseminated tumor cells (CTCs and DTCs), which predict poor outcome in patients and may play a role in tumor progression. However, isolation and detection of human CTCs and DTCs in these xenograft models have been challenging even with EpCAM-based enrichment methods. The goal of this study was to determine if CTCs and DTCs could be identified using human pan-CK staining in a panel of triple-negative primary breast cancer xenograft lines, which could then be employed to study the biology of these cells and to test novel therapies.

**Methods:** We screened 13 stable primary transplantable xenograft lines (1-6 mice per line), established by directly transplanting ethnically diverse triple-negative tumor samples into the epithelium-free mammary fat pads of SCID/Beige mice, for the presence of CTCs and DTCs. The triple-negative status was maintained in these xenograft lines over serial passages. To detect CTCs, peripheral blood mononuclear cells (PBMCs) were isolated from the blood collected from the inferior vena cava either by Ficoll gradient or RBC lysis, with a typical yield of 500,000 PBMCs in 500 μl of blood. Subsequently, PBMCs were immunostained for the presence of CTCs, which were defined as the cells positive for cytoplasmic human pan-cytokeratin staining and nuclear (DAPI/hematoxylin) counter stain. We also flushed the femurs and tibias of 7 xenograft lines to harvest bone marrow cells (BMCs) for the detection of DTCs using the same staining procedure. A xenograft line was considered positive for CTCs or DTCs if they were detected in at least 25% of mice. The presence of lung metastases was assessed in all the xenograft lines by histological examination.

**Results:** We detected CTCs (range: 1-128/20,000 PBMCs) in 6 out of 13 xenograft lines (46%) and DTCs (range: 1-21/20,000 BMCs) in 5 out of 7 (71%) lines. Interestingly, 4 of the 5 DTC-positive lines also had detectable CTCs. High numbers of CTCs (>20/20,000 PBMCs) were found in 3 xenograft lines, one of which also had high numbers of DTCs (>20/20,000 BMCs). No human pan-CK+ cells were detected in PBMCs and/or BMCs from 5 control mice without tumors. Among 13 xenograft lines, lung metastases were found in 5 lines (38%), of which 3 had detectable CTCs or DTCs. Of the 3 xenograft lines containing high CTCs and/or DTCs, 2 had lung metastases.

**Conclusion:** In summary, human pan-CK staining can effectively detect CTCs and DTCs in isolated PBMCs and BMCs of mice bearing triple-negative primary breast cancer xenografts. These xenograft lines with detectable CTCs and DTCs may represent a valuable preclinical model for detailed characterization of human CTCs and DTCs and for the discovery of new therapeutic targets for the triple-negative breast cancer.

**P4-07-04**

**Nomogram Including Circulating Tumor Cells (CTC) Count before and during Chemotherapy for Individual Survival Prediction of Metastatic Breast Cancer Patients.**

**Bidard F-C, Hajage D, Bachelot T, Delaloge S, Brain E, Campone M, Wolp-Diniz R, Dieras V, Mathiot C, Asselin B, Pergo J-Y. Institut Curie, Paris, France; Centre Leon Berard, Lyon, France; Institut Gustave Roussy, Villejuif, France; Institut de Cancérologie de l’Ouest, Nantes, France**

**Background:** CTC count before a new line of treatment and CTC count early changes under chemotherapy have been reported as an independent prognostic marker in metastatic breast cancer in a recent pooled analysis in 841 pts (Liu M. ASCO 2011). The aim of this study was to build a prognostic tool including CTC and other parameters to assess its predictive value for progression-free survival (PFS) and overall survival (OS).

**Methods:** Data from the IC 2006-04 study were used. This prospective multicentre study included 267 metastatic breast cancer patients treated by first line chemotherapy with or without targeted therapy, in whom appropriate pre-treatment prognostic variables (age, performance status, number of metastatic sites, disease-free interval, ER, PR and HER2 status, tumor grade, LDH, serum markers, CTC count by CellSearch technique before treatment and before cycle 2) were available for statistical analysis. We constructed a multivariate Cox regression model for PFS and OS prediction. A stepwise selection process was applied to achieve the most informative and parsimonious models. Performance was measured with the C-index statistic. Internal validation was performed using leave-two-out technique.

**Results:** Four nomograms have been obtained, in two clinical settings: at inclusion (before the start of any treatment) taking into account the initial CTC count, and during treatment (before cycle 2) taking into account CTC changes under treatment. Their accuracy was good for PFS and OS prediction, with C-index ranging from 0.72 to 0.88. Internal validations allow considering a good accuracy of the models in an external population.

**Conclusion:** These clinically relevant nomograms are a simple tool for a personalized prognostic assessment including CTC assessment. Validation on independent series of patients are ongoing.

**P4-07-05**

**Comparison of PIK3CA Hot Spot Mutations in the Primary Tumor or Metastases with PIK3CA Mutations or PIK3CA Over-Expression in Circulating Tumor Cells of Metastatic Breast Cancer Patients under Sequential Palliative Therapy.**

**Aktas B, Kasimir-Bauer S, Kasper S, Derks C, Kimmig R, Schuler M, Tewes M. West German Cancer Center, University Hospital Essen, University of Duisburg-Essen, Essen, Germany**

**Background:** Stem cell like tumor cells have been implied as the active source of metastatic spread in primary tumors. To disseminate and metastasize, these cells may undergo phenotypic changes, known as epithelial-mesenchymal transition (EMT). The PI3K/AKT signaling pathway has been identified as one of the most important and most frequently mutated pathways involved in these processes. Assuming that metastasis requires a dissemination of tumor stem cells or tumor cells showing EMT, we studied 174 blood samples of 43 metastatic breast cancer patients under follow-up of palliative chemotherapy or antihormonal therapy for the presence of stemness like circulating tumor cells (sCTCs). Further, we correlated these data with the occurrence of PIK3CA mutations in sCTCs and with the detection of hot spot mutations as well as PTEN loss in primary tumors or metastases.

**Materials and Methods:** All blood samples underwent immunomagnetic enrichment using the AdnaTest BreastCancerSelect (AdnaGen AG, Germany). RNA was recovered and reverse transcribed for analysis using the AdnaTest EMT (multiplex RT-PCR for TWIST, AKT2, PI3K), and separately for the stem cell marker ALDH1 applying the AdnaTest StemCell. The identification of EMT markers was considered positive if at least one of the three markers was detected in the sample. The expression of CD34 was analyzed in a subset of samples to exclude potential interference of normal hematopoietic stem cells. The analysis of PCR products was performed by capillary electrophoresis on the Agilent Bioanalyzer 2100. The PIK3CA 3140 G/A mutation in sCTC-derived cDNA was identified by direct sequencing. PIK3CA hot spot mutations in primary tumors or
metastases were identified by direct sequencing from microdissected FFPE tumor tissue. PTEN loss (as defined as <10% of cells exhibiting positive staining) was detected by immunohistochemistry.

Results: During follow up, sCTCs were detected in 23/43 (53%) of the patients at least at one time point. ALDH1 was present in 18/43 (42%) patients, and at least one of the EMT markers was detected in 22/43 (51%) of the patients with the over-expression of PI3K (87%), AKT (96%) and TWIST (22%), respectively. Positivity for both, ALDH1 and EMT markers, was found in 15/23 (65%) of the patients. So far, analyzing one sample of each sCTC-positive patient during follow-up of the disease, there seems to be an occurrence of PIK3CA 3140 G/A mutation in sCTCs in about 25% of the patients. Examining the tissue samples, PTEN loss was found in 3/43 (7%) patients, in two of which sCTCs were present. DNA has been extracted from the dissected tumor tissues of all patients and is currently analyzed for PIK3CA mutations. The results will be available for presentation during the SABCS.

Conclusion: This study provides evidence for the presence of therapy-resistant breast cancer stem-like cells in the blood, possibly derived from the tumor or the metastases. Specific PI3K pathway inhibitors, alone or in combination therapy, may provide a therapeutic strategy for eliminating these cells to improve the prognosis of these patients.

P4-07-06
Correlation of Two Analytical Methods for Circulating Tumor Cells in Peripheral Blood of Patients with Primary Breast Cancer.
Jaeger BAS, Rack B, Jueckstock J, Salmen J, Ortman U, Lorenz R, Rezai M, Beck T, Schneeveis A, Zwingers T, Beckmann MW, Friese K, Janni W. Klinikum der Ludwig-Maximilians-Universität - Campus Innenstadt, Munich, Germany; Heinrich Heine-Universitetas, Duesseldorf, Germany; Gemeinschaftspraxis Lorenz-Hecker-Wesche, Braunschweig, Germany; Lisenkrankenhaus, Duesseldorf, Germany; RoMed Klinikum Rosenheim, Rosenheim, Germany; University Hospital Heidelberg, Heidelberg, Germany; Estimate, Augsburg, Germany; Frauenklinik der Universitaet Erlangen, Erlangen, Germany

Background: While the evidence for circulating tumor cells (CTCs) as a prognostic marker in metastatic breast cancer has been well established, there is still a lack of data in primary disease. In the SUCCESS A trial two different techniques for the detection of CTCs in early breast cancer were prospectively evaluated.

Material and Methods: SUCCESS A compared FEC-Docetaxel vs. FEC-Docetaxel-Gemcitabine and 5 vs. 2 years of treatment with zoledronic acid in primary breast cancer patients and node positive or high-risk node negative disease. Two different techniques to detect CTCs were prospectively evaluated in two consecutive, but comparable subgroups of the whole study population.

In 3515 samples the CellSearch® System (Veridex, Warren, USA) was used for CTC detection. Immunomagnetic enrichment with an EPCAM-antibody was followed by labeling with monoclonal antibodies specific for cytokeratin (8, 18, 19) and leukocytes (CD45). 2165 samples were evaluated with a manual immunocytochemistry (MICC) protocol. Cytopsins were prepared after mononuclear cell enrichment based on Oncoquick® centrifugation (greiner bioone, Friekkenhausen, Germany). Staining was performed with the monoclonal pancytokeratin antibody A45-B/B3 (Micromet, Munich, Germany) and the APAAP technique. Conventional light field microscopy (Axiohot; Zeiss, Oberkochen, Germany) was used for the detection of stained cells.

For both methods, the cut-off value for positivity was ≥ 1 CTC. All events were evaluated by two independent observers.

Results: CTCs were examined in a total number of 3243 patients before and after chemotherapy (CHT). The two subgroups evaluated with one or the other method were well-balanced regarding clinical parameters as tumor size, grading, lymph node-status, hormone receptors and Her2. Furthermore there was no significant correlation between the CTC positivity and one of these clinical parameters using CellSearch or the MICC, respectively (p > 0.05 using the chi square test each time).

Before adjuvant CHT 21.3% (424 out of 1994) and 21.1% (264 out of 1249) of the patients were found positive for CTCs using CellSearch® or the MICC, respectively, with a mean CTC level of 5.9 (range: 1 to 827) and 3.1 (range: 1 to 256).

Immediately after CHT 21.9% (333 out of 1521) and 16.5% (151 out of 916) of the patients were positive for CTCs using CellSearch® or the MICC. The mean CTC level decreased to 3.0 (range: 1 to 124) and 2.1 (range: 1 to 23) in both analytical methods.

Using CellSearch® there was a significant correlation between the presence of CTCs before CHT and disease progression (p = 0.0044), as well as survival (p = 0.0001), whereas the MICC did not predict any of these (p = 0.3143 and p = 0.0801 respectively; the chi-square test was used each time).

Conclusion: We found comparable prevalence of CTCs before and after adjuvant chemotherapy both with the CellSearch® System or the MICC. However, prognostic relevance could only be shown for CTCs detected with the CellSearch® System. This may be attributed to the high standardization and reproducibility of the automated system, as well as the additional CD45 counterstaining. According to our findings, the FDA approved CellSearch® System should be used as gold standard for CTC detection in future clinical trials.

P4-07-07
Circulating Tumor Cells Predict Survival in Non-Metastatic Breast Cancer.
Lucel A, Krishnamurthy S, Bhattacharyya A, Lodhi A, Hall C, Singh B, Anderson A, Bedrosian I, Kuerer H. University of Texas MD Anderson Cancer Center, Houston, TX

Background: Circulating tumor cells (CTCs) predict outcome in metastatic breast cancer (BC), but their prognostic significance in non-metastatic BC is unclear. This study determined whether CTCs could be identified in non-metastatic BC patients and 2) if CTCs predict relapse-free and overall survival.

Methods: Clinical stage I-III BC patients seen at a single tertiary cancer center provided informed consent to participate in an IRB-approved study involving collection of blood (7.5 ml x 2 tubes) before systemic therapy and at the time of surgery for their primary BC. CTCs were detected using the Cell SearchTM System. A positive result was defined as the presence of two or more cells per 7.5 ml blood since a threshold for positivity has not been established in primary BC. Statistical analyses were done using STATA-IC 11 software.

Results: We prospectively evaluated 291 patients. Mean age was 54 years, and median follow-up was 30 months. 54% (157) had T1 tumors, 36%(104) T2, 6% (18) T3, and 4%(12) had T4 disease; 37% (107/287) were clinically node-positive (LNs) by axillary ultrasound and FNA. Two or more CTCs were identified in 10% (29) patients. Seventy-seven percent (225) patients were hormone receptor positive, 11% (32) were HER2 positive while 16% (48) expressed no receptors. Sixteen percent (48) were tumor grade 1, 50% (143) were grade 2 and 34% (98) were grade 3. Systemic chemotherapy and/or endocrine therapy were administered in 80% (233/289) of patients. Sixty-eight
percent (167/244) of patients were post-menopausal. Additionally, there was no significant correlation between CTCs with primary tumor size, lymph node status, estrogen or progesterone receptor status, HER2 amplification or tumor grade. Thirty-eight percent (6/16) of all relapses occurred in patients with 2 or more CTCs (HR: 4.48 (95% CI 1.61-12.45), Logrank P 0.002) while 40% (4/10) of all deaths occurred in patients with 2 or more CTCs (HR: 4.54 (95% CI 1.27-16.25), Logrank P 0.011).

Conclusions: CTCs were a significant predictor of disease-free and overall survival in non-metastatic breast cancer.

P4-07-08
Subsets and Molecular Signatures of Circulating Tumor Cells in Breast Cancer Brain Metastasis.
Marchetti D, Zhang L, Wetzel M, Zaidi T, Ridgway W, He W, Groves MD, Katz RL. Baylor College of Medicine, Houston, TX; MD Anderson Cancer Center, Houston, TX

Background: Circulating tumor cells (CTCs) represent the “seeds” of intractable brain metastatic breast cancer (BMBC); however, properties of CTCs targeting the brain remain elusive. For example, the FDA-approved CTC platform (CellSearch™, Veridex, LLC) detects only CTCs positive for epithelial cell adhesion molecule (EpCAM) and cytokeratins (CKs), but is unable to capture any other CTC subtypes or analyze biomarkers of brain-homing CTCs. We hypothesized that profiling CTCs from BMBC patients might result in the identification of brain-colonizing CTC signatures.

Materials and Methods: We employed CellSearch™ and a novel technology that uses analysis of specific antigenic markers by immunofluorescence, coupled with detecting gene amplification by fluorescence in situ hybridization on the same cells; and quantification of the signal via automated scanning (FICTION; BioView Duet-3™ system).

Results: We established that our approach was feasible by performing CTC analyses on peripheral blood mononuclear cells isolated from BMBC patients or patients not possessing overt metastatic disease. We detected a differential gene amplification for human epidermal growth factor receptor1 and 2 (EGFR and HER2, respectively). Second, the number of EpCAM-positive CTCs visualized by the BioView™ platform was at least three orders of magnitude higher than one obtained from CellSearch™ CTC analyses using the same specimen. Third, we identified the presence of CTCs positive for CKs but negative for EpCAM. Conversely, high levels of pro-metastatic heparanase, in conjunction with the expression of aldehyde dehydrogenase-1 (ALDH1), a known cancer stem-cell marker, were detected in CTCs from BMBC patients; with a correlation between heparanase, ALDH1, and high EGFR amplification. Finally, extensive flow cytometric/FACS analyses validated the presence of CTC subsets negative for EpCAM and CD45, a hematolymphoid marker, however enriched for heparanase/ALDH1 expression.

Discussion: These findings indicate that the BioView™ platform not only captures more EpCAM-positive CTCs than CellSearch™ but also allows the detection of novel CTC subtypes possessing varying EpCAM levels. Importantly, they suggest that profiling CTC subtypes in patients with BMBC can be relevant towards the discovery of BMBC founder CTCs. Work is ongoing to further characterize these CTC subtypes, and to assess their abilities to metastasize to brain in xenotransplantation studies using immunodeficient mice.

P4-07-09
Automated Quantitative Assessment of HER2 Expression of Circulating Tumor Cells (CTC) in Metastatic Breast Cancer (IC 2006-04 Study).
Bidard F-C, Ligthart ST, Decraene C, Bachelot T, Delalage S, Brain E, Campone M, Pieta J-Y, Terstappen LWM, Institut Curie, Paris, France; University of Twente, Enschede, Netherlands; Centre Leon Berard, Lyon, France; Institut Gustave Roussy, Villejuif, France; Institut de Cancérologie de l’Ouest, Nantes, France

Background: Beyond quantitative CTC analysis for outcome assessment, their qualitative analysis could predict drug response and resistance. Classification of CTC and quantification of treatment targets on CTC can be subjective as they are morphologically heterogeneous. Current HER2 fluorescence assessment methods rely on a visual comparison between CTC images and external controls (cells lines) and are poorly reproducible. We report an automated classification and quantification of HER2 expression on CTC in a large cohort of metastatic breast cancer (MBC) patients (pts).

Materials and Methods: CTC enumeration and HER2 assessment was performed with the CellSearch® system in pts with MBC included in the prospective IC 2006-04 study (Piera, Ann Oncol 2011), before first line chemotherapy. The FITC conjugated antibody CB11 was used to identify HER2. For quantitative assessment of HER2 the breast cancer lines SKBR3 and MCF7 with known HER2 status were spiked in blood of normal donors and processed with the CellSearch system. Digital images of Cytokeratin-PE, DAPI, CD45-APC, and HER2-FITC from these samples were stored. An algorithm, previously developed in prostate cancer (Ligthart, AACR 2011), was applied on these images to automate identification of CTC. The fluorescence of the HER2 FITC channel for each CTC as well as the CD45+, DAPI+ leukocytes contained within each sample were also quantified using this algorithm. HER2 fluorescence levels of CTC were compared to HER2 status of status of pts. Results: Among 267 pts included in this study, 103 had at least 1 CTC/7.5 ml and HER2 fluorescence assessment. In these pts, correlation analysis between automated (aCTC) and manual (mCTC) CTC counts showed an excellent correlation (R²=0.978). HER2 fluorescence of CTC, varied greatly within and between pts, whereas leukocyte fluorescence was more homogeneous. The vast majority of CTC exhibited less fluorescence than SKBR3 cells, even in HER2+ pts. Primary tumor was HER2+ and HER2- in 36 and 58 pts respectively. Percentage of HER2+ CTC was higher in pts with primary HER2+ status.

## Table 1

<table>
<thead>
<tr>
<th>HER2 status of primary tumor and % of HER2+ CTC</th>
<th>Threshold % of HER2+ CTC within a pt</th>
<th>HER2+ pts that are above this threshold (%)</th>
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Although a rare event, the presence within a sample of at least 1 CTC whose HER2 expression is superior to SKBR3 cells was also strongly associated with HER2+ primary tumor (N=15/36 vs. 3/58; p=0.001).

Conclusion: This study was the first to assess HER2 staining intensity in each CTC isolated from a large number of MBC pts. This was made possible by the aCTC count that was highly correlated with the mCTC count in our cohort and permitted HER2 quantification. Our study shows that HER2 staining is highly heterogeneous among CTC within each pt. These findings demonstrate the feasibility of real time quantitative assessment of treatment targets on CTC and opens a path towards personalized treatment.
P4-07-10 Circulating Tumor Cells in Newly Diagnosed Inflammatory Breast Cancer.
Mego M, Giordano A, De Giorgi U, Hsu L, Lucci A, Dawood S, Woodward WA, Ueno NT, Valero V, Andreopoulou E, Hortobagyi GN, Reuben JM, Cristofanilli M. Comenius University, School of Medicine, Bratislava, Slovakia (Slovak Republic); UT MD Anderson Cancer Center, Houston, TX; Fox Chase Cancer Center, Philadelphia, PA

Background: Inflammatory breast cancer (IBC) is one of the most aggressive forms of breast cancer and is associated with special clinical and biological features. Circulating tumor cells (CTC) are an independent prognostic factor in metastatic breast cancer for progression-free and overall survival. Previously, we showed that metastatic IBC patients treated with first and subsequent lines of chemotherapy had a lower number of CTC when compared with CTC levels of non-IBC patients; hence, the prognostic value of CTC in mIBC patients is limited. In the present study, we investigated the prognostic value of baseline CTC in newly diagnosed IBC patients.

Patients and methods: This retrospective study included 84 newly diagnosed IBC (37 stage III B or C and 47 stage IV) patients treated with neoadjuvant therapy or first line chemotherapy before January 2004 and July 2009 at the MD Anderson Cancer Center. The median age was 55 years (range, 23-78). Twenty-four (28.8%) patients were HER2 positive, 36 (42.9%) hormone receptor positive and 33 (39.3%) were triple receptor negative. CTC were detected and enumerated using the CellSearch® system before patients started chemotherapy.

Progression free survival (PFS) and overall survival (OS) were calculated from the date of CTC measurement and estimated by the Kaplan-Meier product limit method.

Results: At baseline, 64 (76.2%) patients had at least ≥1 CTC and 29 (34.5%) ≥5 CTC. Proportions of patients with stage III IBC with ≥1 CTC and ≥5 CTC were lower than those of newly diagnosed stage IV IBC patients (67.6% vs. 86%; p = 0.12 and 24.3% vs. 42.6%; p = 0.11, respectively). At a median follow up of 22.4 months (range: 4.8 – 58.3 months), 48 (57.1%) patients experienced disease progression and 26 (31.0%) had died. Patients with < 5 CTC had similar PFS (p = 0.36) and OS (p = 0.80) to those of patients with ≥5 CTC, respectively. We investigated the prognostic value of CTC ≥ 1 in stage III IBC patients, as well. There was no difference in PFS (p = 0.71) and OS (p = 0.15) in patients with CTC = 0 vs. those of patients with CTC ≥ 1, respectively. Moreover, we correlated CTC levels with prognosis, using different CTC threshold, but we observed no correlation between baseline CTC and patients’ outcome (Table 1).

Conclusions: CTC can be detected in a large proportion of patients with newly diagnosed IBC; however, baseline CTC was not prognostic for PFS and OS. Collective dissemination of cancer cells with newly diagnosed IBC; hence, the threshold for positivity has not been established in non-metastatic breast cancer.

Statistical analyses used chi-square and Fisher’s exact test.

Results: One hundred and twenty patients were prospectively enrolled. Median age was 50 years and median follow-up was 33 months. Eight percent of patients had T1 disease, 35% T2, 18% T3, and 39% T4. Fifty-three percent of patients (63/120) had hormone receptor positive disease. Thirty-two percent of patients (38/120) were HER-2 positive. Thirty percent (36/120) were triple negative. Seventy-eight percent (91/120) had lymph node positive disease. Two or more CTCs were present in 9% of patients (11/120). Of the 11 patients who died, 3 had 2 or more CTCs (P = 0.08). Of the 20 who relapsed, 6 had 2 or more CTCs (P = 0.0019).

Conclusions: Presence of two or more CTCs after NACT predicted worse relapse free survival in patients with stage I-III breast cancer.

P4-07-11 Circulating Tumor Cells after Neoadjuvant Therapy Predict Outcome in Stage I to III Breast Cancer.
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Introduction: Circulating tumor cells (CTCs) predict outcome in metastatic breast cancer, but their significance is unclear in non-metastatic patients. Furthermore, it is unclear if the presence of CTCs after completion of neoadjuvant chemotherapy (NACT) predicts worse outcome. The purpose of this study was to determine if CTCs after NACT predicts worse outcome.

Methods: Clinical stage I-III breast cancer patients seen at a single tertiary cancer center provided informed consent to participate in an IRB-approved study involving collection of blood (7.5 ml x 2 tubes) at the time of surgery for their primary breast cancer. CTCs were detected using the CellSearch® system. A positive result was defined as the presence of one or more cells per 7.5 ml blood since the threshold for positivity has not been established in non-metastatic breast cancer. Statistical analyses used chi-square and Fischer’s exact test.

Results: At baseline, 64 (76.2%) patients had at least ≥1 CTC and 29 (34.5%) ≥5 CTC. Proportions of patients with stage III IBC with ≥1 CTC and ≥5 CTC were lower than those of newly diagnosed stage IV IBC patients (67.6% vs. 86%; p = 0.12 and 24.3% vs. 42.6%; p = 0.11, respectively). At a median follow up of 22.4 months (range: 4.8 – 58.3 months), 48 (57.1%) patients experienced disease progression and 26 (31.0%) had died. Patients with < 5 CTC had similar PFS (p = 0.36) and OS (p = 0.80) to those of patients with ≥5 CTC, respectively. We investigated the prognostic value of CTC ≥ 1 in stage III IBC patients, as well. There was no difference in PFS (p = 0.71) and OS (p = 0.15) in patients with CTC = 0 vs. those of patients with CTC ≥ 1, respectively. Moreover, we correlated CTC levels with prognosis, using different CTC threshold, but we observed no correlation between baseline CTC and patients’ outcome (Table 1).

Conclusions: CTC can be detected in a large proportion of patients with newly diagnosed IBC; however, baseline CTC was not prognostic for PFS and OS. Collective dissemination of cancer cells via lymphovascular tumor emboli and/or epithelial-mesenchymal transition accompanied by inadequate detection of CTC due to lack of sensitivity of current assays may account for limited prognostic value of CTC in newly diagnosed IBC. Nevertheless, sample size and study power could influence study results as well.

P4-07-12 Identification of p53 Mutation in Whole Genome DNA from Single Circulating Tumor Cells (CTCs) and Primary Breast Cancers (BC) from Patients (pts) with Metastatic Breast Cancer (MBC).

Background: CTCs represent the source of distant metastases, and are also implied in the growth/re-growth of primary BC. Molecular/gene-level characterization of similarities and discords between CTCs and BCs in pts with MBC may provide useful information for individualized treatment. Since somatic p53 mutations are frequently observed in primary BCs, we set out to assess the feasibility of identifying such mutations in CTCs from pts with MBCs, and compare the findings with those of the primary BCs from the same pts.

Material and Method: Fiber-optic Array Scanning Technology (FAST) was used for identification and location of CTCs on large glass substrates. CTCs were identified after blood samples (10 ml) from MBC pts were stained to detect CTCs via automated digital microscopy by morphology, based on immunofluorescence staining for cytokeratin and nucleus, and the absence of CD45. Single CTCs from 10 pts with MBC were identified and removed from the glass substrates. DNA was extracted, and the whole genome of isolated CTCs was amplified by using whole genome amplification method (Sigma). P53 mutations in exons 5, exon 6, exon 7 and exon 8 were assessed. As comparison, genomic DNA from formalin-fixed and paraffin-embedded (FFPE) from primary BCs of the same pts, was amplified using the same method.
Results: p53 mutations were found in 8 out of 10 CTCs, and in 4 out of 10 in primary BC samples. Of 8 mutations detected in CTCs, one silent mutation and 7 missense mutations were seen. One particular point mutation, R181L, previously assessed as functional mutation, was observed in 4 out of 8 CTCs. None of the 4 mutations (a silent mutation, one missense mutation and two different deletions) detected in tumor samples were found in CTCs. We validated that the mutations detected in CTCs were not artifacts occurring during genome amplification, by comparing p53 mutations between unamplified tumor genomic DNA vs. amplified samples.

Conclusion: Whole genome amplification based on extracting DNA from single CTCs using FAST, and identification of mutations such as those in p53, is feasible. The quantitative and qualitative discordance in detecting p53 mutations between CTCs and primary BCs may be due to CTCs acquiring new—as possible epithelial-mesenchymal transition-like-characteristics with metastatic potential as they evolve from the primary tumors or metastatic sites, or, technical issues (analyzing FFPE-preserved vs. CTCs, tumor heterogeneity) may contribute to our findings. Further assessment of the functionality of high frequent functional mutations such as R181L is warranted.

P4-07-13

Mueller V, Riethdorf S, Rack B, Wolfgang J, Fasching PA, Solomayer E, Aktas B, Kasimir-Bauer S, Mury D, Pantel K, Fehm T; University Medical Center Hamburg-Eppendorf; LMU Munich; University Medical Center Düsseldorf; Hospital Erlangen, Friedrich-Alexander University Erlangen Nuremberg, Erlangen; University Medical Center Homburg/Saar; University Medical Center Essen; University Medical Center Tübingen, All Authors on Behalf of the DETECT Study Group

Background: Over the last decade circulating tumor cells (CTC) were established as a prognostic factor in breast cancer patients. However, there are very limited studies comparing different test methods, although several are available. The DETECT trial for metastatic breast cancer patients was designed to investigate the prognostic impact of CTC. Here, we report on the prognostic relevance of CTC testing with different detection methods.

Material and Methods: Patients with primary metastatic breast cancer or metastatic recurrence were prospectively enrolled in this multicenter trial. CTC were detected using the FDA-approved Cell Search® assay applying immunocytochemistry and the RNA-based Adna Test Breast Cancer™. After a median follow-up of 11 months the first survival data are now presented.

Results: Both methods could be performed in 221 patients. Using the CellSearch™ assay 116 of 221 patients were CTC-positive based on the cut-off level of 5 cells. Presence of CTC was associated with the site of metastatic disease. The OS was 15.4 months in CTC positive pts. (95%-CI: 13.5-17.1 mths) compared to 20.4 mths. in CTC negative pts. (19.1-21.9 mths. ; p=0.001). In the multivariate analysis presence of CTC was the only independent predictor for overall survival (HR: 3.4, 95%-CI: 1.7-6.3) including tumourbiological factors, menopausal status, number and sites of metastatic disease. The progression-free survival was not correlated with CTC status in our cohort receiving different types of systemic treatment (p=0.197). When the AdnaTest Breast was performed, 88 of 221 (40%) patients were CTC positive. Except for HER2 status, no correlation could be observed between CTC positivity and any of the clinicopathological factors. CTC positivity assessed by the AdnaTest Breast has no impact on PFS and OS. A multivariate analysis was therefore not performed.

Conclusions: Currently, several different tests are available for CTC detection. Only a few tests have been approved by the FDA and been validated in large clinical trials. Therefore, it will be important to compare new techniques with the Cell Search assay.

P4-07-14
Circulating Tumor Cells (CTCs) Detection and HER2 Profiling by CellSearch® in Non-Metastatic Breast Cancer: An International Ring Study To Assess Inter-Reader Variability.

Ignatiadis M, Pierga J-Y, Campion M, Fehm T, Payne R, Rack B, Mavroudis D, Riethdorf S, Rothe F, Bessi S, Arau CM, Sandri MT, Borgen E, Kraan J, Terstappen LWM, Piccart M, Sotiriou C, Michiels S, Pantel K, Institut Jules Bordet, Brussels, Belgium; Institut Curie, Paris, France; Mayo Clinic, Rochester; Universitèts-Frauenklinik, Tübingen, Germany; Hammersmith Hospital, Imperial College, London, United Kingdom; Ludwig-Maximilians-Universität, Munich, Germany; University of Crete, Crete, Greece; University Medical Center Hamburg-Eppendorf, Hamburg, Germany; Hospital of Prato, Prato, Italy; Vall d’ Hebron, Barcelona, Spain; European Institute of Oncology, Milan, Italy; Oslo University Hospital, Oslo, Norway; Erasmus Medical Center, Rotterdam, Netherlands; University of Twente, Twente, Netherlands

Background: Preliminary results from the Success and Remagrus trials showed that CTC detection by CellSearch® has adverse prognostic value in non-metastatic breast cancer. Moreover, several investigators have characterized HER2 expression on CTCs in early breast cancer. Since the majority of CTC-positive women with non-metastatic breast cancer have only 1 CTC detected/blood volume analyzed, we wanted to evaluate the inter-reader variability in this setting. This is a crucial step before moving forward with multi-lab/multi-center prospective testing of the clinical utility of the CellSearch® technology in non-metastatic breast cancer.

Methods: Five galleries of CellSearch® images from 3 European and one US institution (a total of 307 images) were mailed to 22 independent readers from 14 European and US academic laboratories and 8 readers from two CellSearch® Veridex laboratories in a blinded fashion. These images came mainly from studies on CTC and/or HER2-positive CTC detection in breast cancer and included healthy women (negative controls), women with metastatic (positive controls) and non-metastatic disease (Pierga et al SABCS 2010, Pierga et al ASCO 2011, Riedthorf et al CCR 2010, Ignatiadis et al PLoS ONE 2011). Each reader reported the images as either CTC-negative or CTC-positive/HER2-negative or CTC-positive/HER2-positive. Kappa statistics were used to assess inter-reader agreement. The 8 Veridex readers were summarized by a majority voting system to derive a gold standard in order to compare each independent reader using discordance rates.

Results: Kappa statistics showed moderate to good agreement between independent readers depending on the gallery evaluated (gallery 1 K: 0.55, gallery 2 K:0.57 gallery 3 K:0.64, gallery 4 K:0.72, gallery 5 K:0.85). These differences were attributed to differences in scoring difficulty of each gallery. Discordances in CTC-positive vs CTC-negative events between each reader and the gold standard ranged from 2.3%-31.3% with 7 readers showing discordance rates <5%, 11 readers between 5-10%, 10 readers between 10-14% and only 2 readers showing discordance rates >14% as compared to the gold standard (median discordance rate: 9.3%). Image analysis showed that investigators with high discordance rates compared to the Veridex gold standard were not always taking into account “morphological characteristics” for defining an event as CTC-positive. Discordant
results between readers were mainly due to discordance in CTC definition and to a lesser extent in the definition of HER2-positivity. Indeed, discrepancies in assigning a CTC-positive event as either HER2-negative or HER2-positive ranged from 0.3-13.7% with 19 readers showing discordance rates <5%, 7 readers between 5-10% and 4 readers with discordance rates >10% (median discordance rate: 3.9%).

Conclusion: In non-metastatic breast cancer, we observed low discordance between most independent readers and the gold standard for defining a CellSearch® event as CTC with very few readers showing high discordance rates. Concordance can be improved through appropriate training and the use of all the tools provided by the CellSearch® system for image interpretation.

P4-07-15
Insulin-Like Growth Factor Receptor I (IGFIR) Expression in Circulating Tumor Cells (CTCs) of Patients with Early and Metastatic Breast Cancer (BC).

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Background: Components of the insulin-like growth factor system are deregulated and IGFIR overexpression is commonly observed in BC, however the expression of the receptor in CTCs of patients with BC has not been studied.

Methods: IGFIR expression was assessed in CTCs from 65 patients with early and 101 with metastatic breast cancer before the start of adjuvant and first-line chemotherapy, respectively. Peripheral blood mononuclear cells’ (PBMCs) cytospins were stained with a monoclonal A45-B/B3 anti-cytokeratin (CK) antibody and IGFIR β-subunit anti-rabbit antibody. IGFIR(+)CK(+) CTCs were detected using immunofluorescent microscopy (Ariol system). Phosphorylated IGFIR was also evaluated after staining with phospho-IGFIR-β (Tyr1131)/Insulin Receptor-β (Tyr1146) antibody.

Results: CK(+) CTCs were detected in 23 (35.3%) patients with early and 48.5% with metastatic BC. IGFIR(+) CTCs were identified in all (100%) patients with early and in 38 (78%) out of 49 patients with metastatic BC (p=0.014). Eighteen (78%) out of 23 patients with early disease had exclusively IGFIR(+) CTCs and 5(22%) had both IGFIR(+) and CK(+) CTCs. There were no patients in whom exclusively CK(+) CTCs were identified. The respective values in patients with metastatic disease were 19 (39%), 19 (39%) and 11 (22%) (p=0.004, compared to early disease). The median percentage of the expression of IGFIR(+) and IGFIR(-) CTCs per patient was 100% (range 25-100) and 0% (range 0-75), in early and 68% (range, 0-100) and 32% (range 0-100), in metastatic disease, respectively (p=0.003). A total of 222 and 386 CTCs were detected in patients with early and metastatic disease, respectively. IGFIR expression was observed in 84% and 64% of all detected CTCs in adjuvant and metastatic patients, respectively (p=0.001). Phosphorylated IGFIR has been evaluated in PBMCs’ cytospins from 6 patients with adjuvant and 6 with metastatic disease. Interestingly, among 18 CTCs detected in early BC, all expressed phosphorylated IGFIR, whereas, among 34 CTCs identified in metastatic patients, none expressed the phosphorylated form of the receptor. The evaluation of phosphorylated IGFIR is ongoing and updated results will be presented.

Conclusions: IGFIR expression is commonly observed in CTCs of patients with BC. IGFIR expression is detected in a higher proportion of patients and in a higher proportion of the total number of CTCs identified in early compared to metastatic disease. The above observations suggest that metastatic progression is associated with loss or downregulation of IGFIR expression in breast cancer.

P4-07-16
Development of Circulating Tumor Cell-Endocrine Therapy Index in Metastatic Breast Cancer Patients.

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Introduction: Only ~ 50% of patients (pts) with estrogen receptor (ER) positive metastatic breast cancer (MBC) benefit from endocrine therapy (ET). Currently only clinical judgment can be used to identify pts with endocrine-refractory MBC, who are better palliated with chemotherapy. Circulating Tumor Cells (CTC) are reliably enumerated using an automated immunomagnetic system (CellSearch®; Veridex LLC). High CTC levels predict rapid progression in pts with MBC. We have developed a multi-parameter assay, the CTC-Endocrine Therapy Index (CTC-ETI) using CellSearch® that may identify pts with ER positive MBC who are unlikely to benefit from ET and may be better served with chemotherapy. CTC-ETI scores are assigned based on CTC levels coupled with the relative percent and degree of marker positivity on the CTC. We report preliminary results from a pilot single institutional study.

Methods: CellSearch® has 4 fluorescence channels. Three distinguish CTC from WBC (DAPI, anti-cytokeratin, anti-CD45). The 4th “empty” channel was used to measure ER, BCL-2, HER-2, and Ki-67 expression with fluorescent-labeled antibodies. These 4 markers reflect sensitivity (ER, BCL-2) or resistance (HER-2, Ki-67) to ET. Forty ml of blood was drawn into 4 CellSave® tubes from pts with progressive MBC. Whole blood from 4 tubes was pooled and divided into 4 different 7.5 ml aliquots of blood, which were processed and characterized for CTC counts and each of the four molecular markers using the CXC CellSearch® kit.

Results: 21 pts have been accrued to the feasibility study. One patient was ineligible. Five of 20 pts had low CTC counts (<5 CTC/7.5ml whole blood), and are expected to have a relatively favorable prognosis. CTC-ETI was successfully determined in 10 pts (50%): 2 pts had low, while 3 had intermediate, and 5 had high CTC-ETI. Technical difficulties precluded accurate CTC-ETI in the remaining 5 patients. Of note, expression of the biomarkers among CTC in single patients was heterogeneous, suggesting that future iterations of the CTC-ETI will have to consider expression variability. Further exploratory results regarding associations between CTC-ETI and outcomes will be presented.

Conclusions: ER, BCL-2, HER-2, and Ki-67 can be accurately determined on CTC using the 4th channel in the CellSearch® system to calculate CTC-ETI. We predict that lower CTC-ETI scores (low or no CTC, or CTC with high CTC ER and BCL-2 and low CTC HER-2 and Ki-67) could be associated with favorable response to ET. Successful completion of the feasibility study will lead to a prospective trial to determine if high CTC-ETI at baseline predicts resistance and rapid progression on ET in women starting a new endocrine therapy for MBC.

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P4-07-17
Isolation of Highly Pure Circulating Tumor Cells (CTCs) from Metastatic Breast Cancer (MBC) Patients for Gene Expression Analysis.

Background: The paucity of information regarding the molecular nature of CTCs can be largely attributed to the difficulty of isolating these rare cells. As such, efforts on RNA profiling of CTCs have been limited to studies using immuno-enriched samples containing a high background of leukocytes.

Methods: We developed a two-step process: immunomagnetic enrichment followed by fluorescence activated cell sorting (IE/FACS) to isolate CTCs away from leukocytes and performed expression analysis on a limited panel of genes. Magnetic beads coated with EpCAM mAb were used to enrich for tumor cells. Enriched samples were then subjected to FACS using fluorescently labeled mAbs to distinguish tumor cells (EpCAM+) from leukocytes (CD45+) during sorting. CTCs and matched leukocytes were isolated from 67 sequential patients with MBC. Total RNA from isolated 20-50 cells was reverse-transcribed into cDNA. Sixty four (64) CTC-related genes from previous published reports were chosen for expression analysis. cDNA of these genes were pre-amplified and analyzed in triplicate via Taqman®-based RT-PCR in a low density array format. Statistical analysis of gene expression data was performed using Realtime Statminer®.

Results: Unsupervised hierarchical clustering analysis showed that CTCs clustered away from the leukocytes. Differential gene expression analysis revealed the up-regulation of several genes including EPCAM, MUC1, and KRT19 in CTCs (adjusted p<0.05). In addition, CTCs showed a significant down-regulation of the leukocyte-specific marker PTPRC (CD45) as well as CD44 and VIM, markers associated with stem cellness and epithelial to mesenchymal transition, respectively.

Discussion: We demonstrate the feasibility of isolation and gene expression analysis of CTCs. Molecular profiling confirmed that isolated CTCs were highly pure with little or no evidence of leukocyte contamination. This approach can serve as a non-invasive source of tumor tissue for further molecular characterization of CTCs and for monitoring of therapeutic efficacy of targeted therapies in clinical trials. This study was supported by CALGB and BCRF.

P4-07-18
Prospective Assessment of Circulating Tumor Cells and Serum Markers for PFS Prediction in Metastatic Breast Cancer.
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Purpose: Circulating tumors cells (CTC) have been recently proposed as a new dynamic blood marker whose positivity at baseline is a prognostic factor and whose changes under treatment are correlated with progression-free survival (PFS) in metastatic breast cancer patients. However, serum markers levels are also used for the same purpose, and no clear comparison as been reported to date.

Patients and methods: The IC 2006-04 enrolled prospectively 267 metastatic breast cancer patients treated by first line chemotherapy and confirmed that CTC levels are an independent prognostic factor for PFS and Overall survival (OS). A pre-planned endpoint was to compare prospectively the positivity rates and the value of CTC (CellSearch®), of serum tumor markers (CEA, CA 15-3, CYFRA 21.1), and of serum non-tumor markers (LDH, ALP) at baseline and under treatment for PFS prediction, independently from the other known prognostic factors, using univariate analyses and concordance indexes.

Results: Table 1 shows the incidence of each of the 6 blood markers. Assessing all the 6 markers retrieved 90% of patients with at least one elevated marker at baseline. Interestingly, a combination of two markers (CA 15-3 and CYFRA 21.1, often used in lung cancer) retrieved 86% of patients with at least one marker elevated at baseline. All 6 markers were correlated with poor performance status, high number of metastatic sites and with each other. Each marker was associated, when elevated at baseline, with a significantly shorter PFS in univariate analysis.

Discussion: Markers change during treatment, assessed either between baseline and week 3 or between baseline and week 6-9, were significantly associated with PFS, as reported for CTC. Concordance indexes comparison showed no clear superiority of any of the serum marker or CTC for PFS prediction.

Conclusion: In the largest prospective CTC study in metastatic breast cancer, we previously reported that CTC count, but not serum markers, is an independent prognostic factor for PFS and overall survival. However, for the purpose of PFS prediction by measuring blood marker changes during treatment, currently available blood-derived markers (CTC and serum markers) had globally similar performances. Besides CEA and CA 15-3, CYFRA 21.1 is commonly elevated in metastatic breast cancer and has a strong prognostic value.

P4-07-19
Bone Marrow Involvement Is Associated with High Numbers of Circulating Tumor Cells in Peripheral Blood of Metastatic Breast Cancer Patients.
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Introduction: Circulating Tumor Cells (CTCs) levels are dynamic indicators of prognosis and response to therapy in metastatic breast cancer (MBC) patients (pts). CTCs levels have been reported to be higher in pts with bone than visceral metastases, respectively. However, little is known about CTCs levels in pts with BM involvement. We analysed CTC patterns in patients with either visceral, bone or BM to test the hypothesis that BM involvement would facilitate tumor cells to enter the systemic circulation as CTCs. Patients and methods: Sites and extension of metastatic disease were identified with conventional imaging. Bone metastases were diagnosed with either bone scintigraphy or CT-PET and the BM involvement was documented histologically by BM biopsy and metabolically by CT-PET. A training set of 10 pts was evaluated by both BM biopsy and CT-PET to verify the diagnostic performance of CTC-PET in identifying BM metastases (M+). We found a 100% concordance between BM biopsy and CT-PET and we therefore used CT-PET for further analyses. The CellSearch System (Veridex LLC, Raritan, NJ, USA) was used for the isolation and enumeration of CTCs in peripheral blood. The Student’s t-test and the two-way ANOVA test were used to compare means of CTCs among patients with different metastatic patterns.
Results: The CTCs mean and median scores of 129 MBC pts were analysed. Final analysis focused on 101 pts with bone M+. Thirty-three pts had bone M+ alone and 68 pts had bone and visceral M+ (including lymphnodes, brain, liver and lung/pleura). Number of metastatic sites was 1, 2, 3, 4, 5 in 30%, 37%, 25%, 6% and 2% of pts, respectively. BM involvement was documented in 27 (27%) of pts in the whole series and in 12 (33%) of pts with bone M+ alone. Mean CTCs score in the whole series was 392 (0-9993) and the median number of CTCs was 20. In the whole series, BM involvement was associated with higher mean CTCs scores (1350 vs 42, p = 0.014). In pts with bone M+ alone, mean CTCs score was 696 (0-7000) and pts with BM involvement had higher mean CTCs scores (1846 vs 39, p = 0.37). Among the different metastatic patterns, the association of bone with BM involvement and liver M+ was correlated with the highest CTCs numbers. We could not perform comparisons in the 68 pts with both bone and visceral localizations because of the variety of metastatic patterns. However, the two-way ANOVA showed a significant influence of BM involvement on mean CTCs scores in pts with different metastatic patterns (p = 0.001).

Conclusions: This work confirms that pts with bone metastases have higher numbers of CTCs than pts with visceral metastases. Extensive bone disease with BM involvement is characterized by an increase in CTCs numbers compared to bone disease without BM involvement. Bone marrow metastases are metabolically more active by CT-PET and can be accurately detected by metabolic imaging without need of BM biopsy. Further studies are ongoing.

P4-07-20
Apoptosis in Circulating Tumor Cells (CTCs) of Early and Metastatic Breast Cancer Patients.
Kallergi G, Konstantinidis G, Papadaki M, Agelaki S, Mavroudis D, Stournaras C, Georgoulas V. School of Medicine, University of Crete, Heraklion, Crete, Greece; University General Hospital of Heraklion, Heraklion, Crete, Greece.

Background: Metastasis has been associated with the presence of circulating (CTCs) and disseminated (DTCs) tumor cells in peripheral blood and bone marrow of breast cancer patients, respectively. Recent studies have confirmed that the detection of CTCs and DTCs represents a strong and independent prognostic factor for decreased disease-free and overall survival. However, it is not clear so far whether all CTCs are capable to generate metastasis or some of them are destined to die. The aim of the present study was to analyze the presence of apoptotic CTCs in patients with early and metastatic breast cancer. Patients and methods: Double staining immunofluorescent (IF) experiments were performed in peripheral blood mononuclear cells (PBMC) cytospins of patients with early (n=23) and metastatic breast cancer (n=29), prior to the initiation of adjuvant and first-line chemotherapy, respectively. Pancytokeratin A45-B/B3 antibody (as a marker of CK-positive cells) was coupled with either M30 (marker of apoptotic cells) or Ki67 (marker of proliferating cells) antibodies. Apoptotic CTCs were also evaluated using a polycaspase detection kit. Results: Significantly lower proportions of apoptotic CTCs were detected in metastatic compared to early breast cancer patients using the polycaspase kit (3% vs 49%, p = 0.0001). These results were confirmed with M30 staining (32% vs 67%, in metastatic and early disease, respectively, p = 0.023). The median percentage of apoptotic CTCs per patient was also lower in advanced compared to early disease patients (0% vs 100% with the polycaspase kit and 15% vs 70% with M30 staining). Ki67 positive CTCs were identified in 56% of early and metastatic patients, while the ratio of Ki67-positive/Total CTCs was 15% and 36% respectively. Conclusions: Apoptotic CTCs are more commonly observed in early compared to metastatic breast cancer, whereas, Ki-67 positive CTCs are more frequently encountered in metastatic disease. The differential incidence of apoptotic and proliferative CTCs in early and metastatic disease is probably related to disease progression. The evaluation of these markers in CTCs from patients with breast cancer may provide useful prognostic information and could be used to monitor the effectiveness of therapy.

P4-07-21
A Comparison of Two Methods for the Detection of Circulating Tumor Cells (CTCs) in Patients (pts) with Early and Metastatic Breast Cancer (BC): RT-PCR for Cytokeratin (CK) -19 mRNA Versus the CellSearch System.
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Background: Different methods are available for the detection of CTCs in pts with BC, however, the variable performance of these assays, the heterogeneity of CTCs, and the possible treatment-induced alteration of the markers evaluated, imply that no ideal method currently exists. We compared the efficiency of two methods to detect CTCs in pts with BC.

Patients and Methods: Blood was obtained from 200 pts with early and 164 with metastatic BC before the start of adjuvant or first-line chemotherapy, respectively. Different aliquots of the same sample were evaluated by RT-PCR for CK-19 mRNA and by the CellSearch System. Blood samples were available after the end of adjuvant or first-line therapy in 99 and 93 pts, respectively. CTCs in 23 and 7.5 ml of blood were enumerated by CellSearch, in adjuvant and metastatic pts, respectively. Cut-off values of ≥ 1 and ≥ 2 CTCs/23 ml and ≥ 2 and ≥ 5 CTCs/7.5 ml were used. Twenty ml of blood were obtained for mRNA extraction in all RT-PCR experiments.

Results: In early BC, 18.0% of samples were positive prior to therapy by RT-PCR and 37.0% (CTC≥ 1) and 16.5% (CTC≥ 2) by CellSearch. Blood samples were available after the end of adjuvant or first-line therapy in 99 and 93 pts, respectively. CTCs in 23 and 7.5 ml of blood were enumerated by CellSearch, in adjuvant and metastatic pts, respectively. Cut-off values of ≥ 1 and ≥ 2 CTCs/23 ml and ≥ 2 and ≥ 5 CTCs/7.5 ml were used. Twenty ml of blood were obtained for mRNA extraction in all RT-PCR experiments.

Conclusions: This work confirms that pts with bone metastases have higher mean CTCs scores (1846 vs 39, p = 0.001). Overall agreement was 60.6% (CTC≥ 2) by CellSearch and 11.6% by RT-PCR (Spearman, R = -0.031, p = 0.761). Overall concordance was 62% for CTC≥ 2 (Chi-Square, p = 0.161 and p = 0.307). Post-chemotherapy, the positivity rate was 33.6% (CTC≥ 1) and 18.8% (CTC≥ 2) by CellSearch and 11.6% by RT-PCR (Spearman, R = -0.031, p = 0.761). Overall agreement was 60.6% (CTC≥ 1) and 71.4% (CTC≥ 2) (Chi-Square, p = 0.771 and p = 0.708). In the metastatic setting, 37.8% of the samples were positive by RT-PCR, and 50% (CTC≥ 2) or 32.3% (CTC≥ 5), by CellSearch (Spearman, R = 0.373, p = 0.0001). Overall agreement was 70.0% for CTC≥ 5 (Chi-Square, p = 0.0001). Post-chemotherapy, 21% were positive by RT-PCR and 13.7% and 8.4% by CellSearch for CTC≥ 2 and ≥ 5, respectively (Spearman, R = -0.194, p = 0.063). Overall concordance for CTC≥ 5 was 79.6% (Chi-Square, p = 0.017). Agreement was also observed for CTC≥ 2, pre-and post-chemotherapy (Chi-Square, p = 0.0001 and p = 0.010). The concordance rates in the adjuvant and metastatic settings are shown in Table 1.
Predictive Value of HER2 Serum Levels in Patients Treated with Lapatinib or Trastuzumab – A Translational Project in the Neoadjuvant “Geparquinto” Trial.

Witzel ID, Loibl S, von Minckwitz G, Abdallah A, Kähn T, Overkamp F, Fehm T, Schrader I, Ueber C, Kohls A, Stürbmer S, zu Eulenburg C, Unnich M, Müller V. University Medical Center Hamburg-Eppendorf, Hamburg, Germany; German Breast Group, Neuss, Germany; Helios Klinikum Berlin Buch, Berlin, Germany; University Medical Center Tübingen, Tübingen, Germany; Hospital Gelsenkirchen, Gelsenkirchen, Germany; Hospital Esslingen, Esslingen, Germany; Oncologianova, Recklinghausen, Germany; Gynäko-Onkologische Praxis Hannover, Hannover, Germany; Gynäkologische Gemeinschaftspraxis, Hildesheim, Germany; Ev. Krankenhaus, Ludwigsfelde-Teltow, Germany; Frauenklinik Rheinfeld, Rheinfeld, Germany

Background: Neoadjuvant chemotherapy (NT) has become an important approach to assess therapeutic efficacy of new treatment strategies. We observed a predictive role of serum HER2 (sHER2) with neoadjuvant trastuzumab treatment in the previous trial “Geparquattro”. Predictive markers for response to new HER2-targeted therapies are still lacking. Therefore, we investigated the role of sHER2 in the context of trastuzumab and lapatinib treatment.

Methods: The clinical trial Geparquinto incorporated either trastuzumab or lapatinib treatment combined with chemotherapy for HER2-positive breast cancer patients. Serum samples were taken at three different time points: before initiation of NT, after 4 cycles of chemotherapy with epirubicin and cyclophosphamide, and after finalization of NT with 4 cycles of docetaxel (pre-surgery). Only those patients were included in the analysis with serum available at all three time points. sHER2 levels were measured by a commercially available ELISA in 159 patients with a HER2-positive primary tumor, 77 (48%) patients were treated with trastuzumab, 82 (52%) with lapatinib. Pathological complete remission (pCR) was defined as no microscopic evidence of invasive residual tumor cells in the breast and lymph nodes.

Results: Overall pCR rate in this cohort was 28%. sHER2 levels were higher in patients with larger tumors (>5 cm), p=0.012) and positive nodal status (p=0.001). Higher pre-chemotherapy sHER2 levels were associated with higher pCR rates in the entire study cohort (OR 2.9, 95% CI 1.3-6.7, p=0.012). Additionally, in the lapatinib treated patient group, a decrease of serum levels of more than 20% after 4 cycles of chemotherapy showed a tendency to be associated with higher pCR rates (OR 7.7, 95% CI 0.8-70, p=0.071). In contrast to the finding of the previous trial Geparquattro, this was not the case in the trastuzumab-treated patient group (p=n.s.).

Conclusions: Results of this study demonstrate pre-chemotherapy sHER2 levels to be an independent predictor of response to NT with both trastuzumab and lapatinib treatment.

P4-08-02
Estradiol (E2) Mediated Secretion of Vascular Endothelial Growth Factor (VEGF) and Stimulation of T-Regulatory Cells (T-Regs): Their Role for the Worse Prognosis of Breast Cancer (BC) in Premenopause.

Roccia F, Candeloro G, Desideri G, Necoziene S, Recchia COC, Rea S. Civilized Hospital, Avezzano, AQ, Italy; University, L’Aquila, AQ, Italy

Background: E2 plays a key role in all the phases of human reproduction, including promotion of uterine basal membrane erosion, embryo implantation, placental villi development, and pregnancy maintenance. E2 mediates all these functions through the induction of VEGF secretion and stimulation of T-Regs with increased tolerance for the eutrophic embryo. BC might utilize the same pathways for its growth and for disease progression: Modulation of VEGF expression in BC cells through transcriptional activation and regulation of the peripheral development of CD4+CD25+ T-Regs cells. Both VEGF and T-Regs are important prognostic factors: In fact high levels of VEGF expression are associated with significantly worse survival in breast cancer patients, even in sub-groups expected to have a better prognosis and T-Regs quantification in breast tumors is valuable for assessing disease progression and prognosis. However few clinical studies investigated the association between E2, VEGF and T-Regs in humans. Objective of this study was to evaluate whether E2 suppression with an LH-RH analogue was able to down regulate VEGF expression, and decrease T-Regs in premenopausal patients with high-risk estrogen receptor positive (ER+) and negative (ER-) early BC. Material and Methods: From 04-2003 to 10-2008, 100 premenopausal early BC patients were entered into the study. At baseline, in the follicular phase of the menstrual cycle, after surgery, plasma E2, VEGF and T-Regs were measured. Measurements were repeated every six months. Treatment: LH-RH analogue for 5 years, chemotherapy tailored to the biological characteristics of each patient, radiation therapy, and 5-year hormonal therapy in ER+ tumors. Primary end-point was the evaluation of VEGF and T-Regs. Secondary end points were progression-free survival (PFS) and overall survival (OS). Results: Median age was 43 years (range 26-45). Median number of positive axillary nodes was 3. Thirty-eight-three patients were ER+, 17 were ER- and progesterone receptor negative (PGR-), 10 were triple negative, 20 were Herb-2 positive. Median Ki-67 was 33% (range 15%-100%). A statistically significant decrease of E2, VEGF and T-Regs was observed after 1 and 5 years, in ER+ and ER- patients. In particular, VEGF (P<0.001) and T-Regs (P<0.001) decreased in 95% of patients that were disease-free. No unexpected toxicity of chemotherapy was observed, while hot flashes and G1 osteopenia occurred after LH-RH analogues administration. After a median follow-up of 55 months (range 30-95), 5-year PFS and OS rate were 95% and 100%, respectively. Discussion: E2 deprivation with an LH-RH analogue is able to decrease plasma VEGF and T-Regs levels in premenopausal high risk ER+ and ER- BC patients. These data show how E2, through VEGF and T-Regs modulation, may be responsible for the worst prognosis that is observed in premenopausal BC.
Serum Autoantibodies to Breast Cancer Associated Antigens Reflect Tumor Biology: An Opportunity for Early Detection & Prevention?

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Autoantibodies (AABs) are produced as an immune response to abnormal (‘non-self’) cancer antigens. Previous studies have reported that AABs can be measured in the blood long before cancers are presently diagnosed, e.g., up to 4 years before screening mammography identified breast cancers and up to 5 years before screening CT detected lung cancers. EarlyCDT™-Lung is currently available as an aid to early detection of lung cancer in high risk patients and measures a panel of seven AABs to general cancer antigens and also lung cancer (LC) specific antigens. These AABs have previously been reported to be associated with the two main types of LC i.e., non-small cell and small cell LC. This study looked at AABs to 4 general cancer antigens to evaluate whether their levels reflected different biology in primary breast tumors.

Methods
770 patients presented with primary breast cancer to three centers (Nottingham, UK n=323; Munich, Germany n=320; Oklahoma, USA n=127); the median ages and ranges were 61 (26-82), 61 (20-88) & 65 (54-84) years, respectively. All had serum samples taken post-diagnosis and pre-treatment. The tumors were well characterized for histological grade, estrogen receptor (ER), progesterone receptor (PgR) and HER2 status. Serum samples were tested for AABs to four generic cancer antigens (Aggs) (p53, SOX2, NY-ESO-1 and Annexin1) originally included as part of Oncimmune’s EarlyCDT™-Lung assay. The AABs were measured by ELISA on the Oncimmune platform, and the EarlyCDT™-Lung cutoffs were used to determine positivity.

Results
131/770 (17%) of primary breast cancers showed elevated AAB levels to one or more of the limited panel of four generic antigens. Positivity for each AAB was correlated with histological grade, ER, PgR and HER2 status. The results, which were similar for each of the three centers, were combined, and the results are shown in Table 1 below.

Table 1.

<table>
<thead>
<tr>
<th>AAB</th>
<th>High Grade</th>
<th>ER positive</th>
<th>PgR positive</th>
<th>HER2 positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>p53</td>
<td>17/19 (89%)</td>
<td>7/10 (70%)</td>
<td>4/6 (67%)</td>
<td>7/12 (58%)</td>
</tr>
<tr>
<td>NY-ESO-1</td>
<td>17/19 (89%)</td>
<td>7/10 (70%)</td>
<td>4/6 (67%)</td>
<td>7/12 (58%)</td>
</tr>
<tr>
<td>SOX2</td>
<td>18/20 (90%)</td>
<td>8/9 (89%)</td>
<td>6/10 (60%)</td>
<td>8/10 (80%)</td>
</tr>
<tr>
<td>Annexin 1</td>
<td>9/11 (82%)</td>
<td>5/6 (83%)</td>
<td>3/5 (60%)</td>
<td>6/8 (75%)</td>
</tr>
</tbody>
</table>

p53 AAB positive cancers tended to be hormone receptor negative and HER2 positive. NY-ESO-1 positive tumors were almost all higher grade with the majority hormone receptor and HER2 negative. SOX2 positive cancers tended to have a hormone sensitive phenotype (i.e., hormone receptor positive and HER2 negative). Annexin1 positive cancers also tended to have a hormone sensitive phenotype as well as HER2 negative. The pattern was statistically different for the four AABs (p<0.001). The autoantibody profile for ER positive tumors was not statistically different from PgR positive tumors.

Conclusions
These data show that specific AABs measured in the serum reflected the biology of the breast cancers. Confirmation of this finding could, in the future, lead to using immuno-biomarkers such as these to guide early therapeutic intervention (e.g. prevention) in a targeted group of women.
P4-08-05
Diagnostic Significance of Exosomal miRNAs in the Plasma of Breast Cancer Patients.

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Background and Aims: Emerging evidence that microRNAs (miRNAs) play an important role in cancer development has opened up new opportunities for cancer diagnosis. Recent studies demonstrated that released exosomes which contain a subset of both cellular mRNA and miRNA could be a useful source of biomarkers for cancer detection. Here, we aim to develop a novel biomarker for breast cancer diagnosis using exosomal miRNAs in plasma.

Methods: We have developed a rapid and novel isolation protocol to enrich tumor-associated exosomes from plasma samples by capturing tumor specific surface markers containing exosomes. After enrichment, we performed miRNA profiling on four sample sets: (1) Ep-CAM marker enriched plasma exosomes of breast cancer patients; (2) breast tumors of the same patients; (3) adjacent non-cancerous tissues of the same patients; (4) Ep-CAM marker enriched plasma exosomes of normal control subjects. Profiling is performed using PCR-based array with human microRNA panels that contain more than 700 miRNAs.

Results: Our profiling data showed that 15 miRNAs are concordantly up-regulated and 13 miRNAs are concordantly down-regulated in both plasma exosomes and corresponding tumors. These account for ~25% (up-regulation) and ~15% (down-regulation) of all miRNAs detectable in plasma exosomes. Our findings demonstrate that miRNA profile in EpCAM-enriched plasma exosomes from breast cancer patients exhibit certain similar pattern to that in the corresponding tumors. Based on our profiling results, plasma signatures that differentiated breast cancer from control are generated and some of the well-known breast cancer related miRNAs such as miR-10b, miR-21, miR-155 and miR-145 are included in our panel list. The putative miRNA biomarkers are validated on plasma samples from an independent cohort from more than 100 cancer patients. Further validation of the selected markers is likely to offer an accurate, noninvasive and specific diagnostic assay for breast cancer.

Conclusions: These results suggest that exosomal miRNAs in plasma may be a novel biomarker for breast cancer diagnosis.

P4-09-01
Identification of Poor Prognosis T1T2N0 Luminal ERBB2-ve Breast Carcinomas.


Background: To identify ER+ve ERBB2-ve duc tal T1T2N0 carcinomas associated with a poor prognosis remains challenging. We have previously demonstrated that the number of chromosomal breakpoints assessed by CGH could be a marker of worse outcome for breast carcinomas. Our aim was to validate the CGH based signature in a series of luminal duc tal and T1T2 N0 carcinoma patients with long-term clinical follow-up. Patients and methods: We analyzed 214 patients treated for an invasive ductal ER+ve ERBB2-ve carcinomas, smaller than 30mm. The training set was composed of 109 patients (10.9 years of median follow-up; 30 cases associated with a metastatic event within less than 4 years/79 control cases with no metastasis event at 5 years) and the validation set of 105 patients (10.5 years of median follow-up, 30 relapses including contra-lateral breast carcinomas, loco-regional relapses and 8 metastatic events). None of the patient received adjuvant chemotherapy. 16 received an adjuvant hormonotherapy (10 in the training and 6 in the validation groups). We genotyped the sample set with the SNP6.0 affymetrix array. After RNA normalization using Genotyping console, segmentation was performed according to the Zhang and Siegmund maximum method. In the training data set, the number of breakpoints was assessed, linked to outcome and the threshold optimising the sensitivity and specificity was determined (ROC curve). The threshold prognostic value was then tested on the validation series (Kaplan Meier analysis, log rank test, determination of relative risk and its confidence interval with a Cox model). Results: In the training set, median numbers of breakpoints were 7 in cases that experienced a metastatic event after more than 5 years and 40.5 in cases that experienced a metastatic event in less than 4 years. The threshold (Younden index) was 34 breakpoints with a sensitivity of 0.57 and a specificity of 0.94 (AUC: 0.81[0.71;0.91]). In the validation set, the outcome of patients with more than 34 breakpoints was poorer than that of patients with less than 34 breakpoints (<34 breakpoints: 19 events in out of 83 patients; >34 breakpoints: 11 events out of 22 patients with a median time to progression of 108 months; p=0.001 (logrank test); RR: 3.7 [1.73; 7.92]). In multivariate analysis, the number of breakpoints (>34 versus <34) remained the only significant parameter for prediction of outcome (RR: 3.12, CI[1.33; 7.31]. p = 0.009). Histoprostagrade, significant in univariate analysis, was not significant in multivariate analysis but was correlated with the number of breakpoints. The number of breakpoints was statistically significant for prediction of metastatic free interval (<34 breakpoints: 4 events in out of 83 patients; >34 breakpoints: 4 events out of 22 patients with a median time to progression of 108 months; p=0.009 (logrank test); RR: 5.29 [1.32; 21.26]). Conclusion: We demonstrated that patients with T1T2 (<3cm) N0 ER+Ve ERBB2-ve invasive duc tal carcinomas harboured a shorter disease free and metastatic free intervals based on genomic profiles assessed by SNP6.0 with a threshold of more than 34 breakpoints. This new approach to assess prognosis in luminal carcinomas is based on a single genomic platform, could allow identification of future therapeutic targets.

P4-09-02
G Protein-Coupled Estrogen Receptor 1 Positively Correlates with Estrogen Receptor α Expression and Increased Distant Disease-Free Survival of Breast Cancer Patients.


Background: Endocrine therapy is an important therapeutic choice for patients with estrogen receptor (ER)-α-positive breast carcinoma and functions by blocking proliferative estrogen signalling through the classical nuclear ERα. G protein-coupled estrogen receptor 1 (GPER1) is a novel membrane estrogen receptor responsible for unique estrogen responses in vitro and in vivo and that is activated by tamoxifen. The aim of this study was to determine the correlation of GPER1 with clinicopathological variables and distant disease-free survival (DDFS) in breast cancer patients treated with and without adjuvant tamoxifen, and whether the prognostic impact is dependent on ERα-status.

Material and Methods: GPER1 was investigated by immunohistochemistry in tissue microarrays of breast tumors from 208 premenopausal node-negative patients (median age 47 years; range 30-57), mainly (87%) not subjected to any adjuvant systemic treatment, and from 273 stage II patients (median age 63 years; range...
26-81), all treated with adjuvant tamoxifen for 2 years. Because almost 90% of the samples had a high percentage (>50%) GPER1-stained cells, we decided to evaluate only the staining intensity (negative, very weak, weak, moderate, and strong) for this variable. Pearson’s χ2 test for trend was used for analyzing association between GPER1 and categorical clinicopathological variables, a test for trend based on ranks for association with age and tumor size, and a log-rank test for trend for evaluating the impact of GPER1 on DDFS after 5 years of follow-up.

Results: GPER1 positively correlated with ERα (P=0.0005 and P=0.01, respectively) and progesterone receptor expression (P=0.004 and P=0.01, respectively) in both the premenopausal and tamoxifen-treated groups, but not with HER2 expression (P=0.45 and P=0.42, respectively). In the premenopausal group, GPER1 negatively correlated with tumor size (P=0.02) and positively with age (P=0.003), whereas in the tamoxifen group GPER1 did not correlate with either tumor size or age. During 5 years of follow up, 64 patients were diagnosed with distant recurrences in the tamoxifen group and 34 patients in the premenopausal group. In univariate analysis, GPER1 positively correlated with DDFS in the tamoxifen group (P=0.04), but non-significantly in the premenopausal group (P=0.08). When stratifying for ERα-status, GPER1 was a prognostic factor in the ERα-positive subgroup (P=0.02 in tamoxifen group and P=0.08 in premenopausal group), but not in the ERα-negative subgroup (P=0.57 and P=0.95, respectively).

Conclusion: We propose that GPER1 is a prognostic marker for increased DDFS in ERα-positive breast cancer. While our results suggest that GPER1 is also a tamoxifen-predictive factor, this needs to be further studied, ideally in a randomized trial comparing clinical outcome for patients treated with and without adjuvant tamoxifen.

P4-09-03
Clinical Significance of HER2+ and Triple-Negative Status in Patients with Tumor Size ≤ 1 cm and Node Negative Breast Cancer.
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Background: Data regarding the clinical significance of HER2 and triple negative status in patients with small node negative tumors is limited. Several small case series have suggested an increased risk of recurrence in patients with T1abN0M0 HER2+ or triple negative (TN) tumors. Conversely, a recent study reported that the recurrence risk for T1abN0M0 HER2+ tumors was too low to justify any adjuvant chemotherapy or trastuzumab. Therefore it remains unclear who among those with small lesions might possibly benefit from more aggressive adjuvant therapy.

Methods: We identified all node negative breast cancer patients with tumor size ≤ 1 cm diagnosed between 1/1/1995 and 12/21/2008 through our institutional breast service database. Patients were classified according to their receptor status into three groups: 1) Hormone receptor (HR)+ (ER or PR positive, HER2 negative); 2) HER2+ (IHC 3+ or FISH ≥2); and 3) TN (ER, PR, and HER2 negative). Disease-free survival (DFS) was calculated using Kaplan-Meier methods. Cox proportional hazards models were used to estimate the association between breast cancer subtypes with DFS adjusting for patient and disease characteristics.

Results: Among 658 patients with tumor ≤ 1 cm, 494 (75%) of the patients were HR+, 109 (17%) were HER2+, and 55 (8%) were TN. Two hundred thirty-two tumors were classified as T1a, and 426 were classified as T1b. Median age was 58 years (27-92). Median follow-up was 3.5 years. The 5-year DFS rates were 97.9%, 95.6%, 93.5% in patients with HR+, HER2+, and TN, respectively (P=0.026).

In multivariate analysis, TN status was associated with worse DFS (HR 3.13, 95% CI 1.26-7.73), while HER2+ was not (HR 1.64, 95% CI 0.73-3.69). In the HER2+ group, 5-year DFS was also not different between patients who received chemotherapy +/- trastuzumab (32%) and those who did not (P=0.588). We also analyzed T1aN0 and T1bN0 patients separately in order to differentiate the sub-populations of patients more likely to benefit from systemic adjuvant chemotherapy. TN status remains an independent risk factor for worse DFS in T1bN0 (HR 3.68, 95% CI 1.17-11.6), but not in T1aN0 patients.

Conclusion: TN, but not HER2+ status was associated with worse DFS in patients with T1aN0M0 tumors, and adjuvant chemotherapy should be considered in TN breast cancer patients who have T1bN0 tumors.

P4-09-04
Do Decreased CEP17 Signals Indicate the Presence of “Monosomy” in Breast Cancer? A Study of HER2 Gene Status and HER2 Immunohistochemistry.
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Background: Detection of HER2 gene amplification by fluorescence in-situ hybridization (FISH) is one of the key tests predicting clinical response of breast cancer to trastuzumab. According to the recent ASCO/CAP guidelines for HER2 testing, the tumor is considered positive for HER2 gene amplification when the HER2/CEP17 ratio is greater than 2.2, negative if less than 1.8, and equivocal if the ratio is between 1.8 and 2.2. The need to use CEP17 as reference for HER2 assumes that the copy number of CEP17 is equivalent to “polysomy”. This study aims to examine the effect of decreased CEP17 signals interpreted as “monosomy” on HER2 protein expression and gene amplification, and to test the hypothesis that a subset of these cases may be inappropriately classified as amplified or equivocal.

Material and Methods: A total of 57 breast cancer cases were tested for HER2 gene status and HER2 Immunohistochemistry. 45 cases were stained in parallel by IHC and FISH and were selected based on HER2 signals > 2 and CEP17 signals < 1.5. To further examine chromosome 17 copy number, FISH mapping studies were performed on 5 cases, using probes for SMS, RARA, and TP53 loci which are telomeric to the CEP17 and HER2 loci. Chromosome 17 status was determined by correlating the mean copy numbers of HER2, CEP17, SMS, RARA, and TP53. A gene locus with signal number/cell between 1.4 and 2.4 was considered consistent with eusomy.

Results: Of the 57 cases, 54 were included in the HER2 ≥ 2 group (457s). Of these 54 cases, 49 cases tested positive for HER2 amplification when the HER2/CEP17 ratio was greater than 2.2, 3 cases were negative if less than 1.8, and 2 cases were equivocal if the HER2/CEP17 ratio was between 1.8 and 2.2. We performed additional FISH hybridization (FISH) to alternative chromosome 17 genes (SMS, RARA, and TP53). The number of CEP17 signals/cell of these 5 cases ranged from 1.01 to 1.15 and the HER2 signals/cell ranged from 2.05 to 2.84. In these cases when additional probes to
chromosome 17 were used as reference genes, 4/5 cases remained amplified or equivocal. One case with HER2 signals of 2.05 changed from equivocal to negative. 4/5 of these cases showed equivocal IHC (2+).

Discussion:
Application of strict ASCO-CAP guidelines on 59 “monosomy” cases showed that all were either amplified or equivocal by FISH, with the vast majority showing equivocal (2+) IHC. In cases with decreased CEP17 signals, most cases remained as “monosomy” using the alternative chromosome 17 reference genes. This may be due to deletion of the short arm of chromosome 17 or true monosomy of chromosome 17. Further studies are warranted on the cases with 2-4 HER2 signals per cell and “monosomy” in order to permit accurate determination of HER2 gene status and appropriate treatment with anti-HER2 therapy.

P4-09-05
CA15-3 Adds Prognostic Information in “Luminal” Type Breast Cancer – A Single Center Experience Evaluating 700 Patients with a Median Follow-Up of 5 Years.
Kantelhardt EJ, Vetter S, Steer S, Ruider T, Holzhausen H-J, Strauß H-G, Große R, Thomassen C, GYN/OB, Halle (Saale), Germany; Inst. of Pathology, Halle (Saale), Germany

Background: Prognostic markers are essential for the decision about individual therapy for patients with newly diagnosed breast cancer. Biological meaningful cancer types are revealed by gene expression analysis. Steroid hormone receptor (HR) and HER2 status of the tumor by immunohistochemistry (IHC) are more easily available and predominantly resemble these cancer types. The “luminal” type as HR pos. and HER2 neg., the “luminalHER2” type as HR pos. and HER2 pos., the “HER2” type as HR neg. and HER2 pos. and the “triple neg.” type as HER neg. and HER2 neg. were evaluated. Preoperative serum CA 15-3 and CEA within IHC-cancer types and outcome using our own cohort from 1999-2010.

Material and Methods: Since 1999 all patients with breast cancer were entered in our tumor registry. Patients were treated by surgery and adjuvant therapy according to national guidelines (www.ago-online.de). Data was entered into SPSS by a specially trained study nurse. Follow-up was obtained yearly using our own out-patient clinic, information from general practitioners and the general cancer registry. Informed consent was taken from the patients at time of diagnosis. CA 15-3 and CEA were defined elevated if above 25 U/ml or 4.6 µg/l respectively.

Results: Preoperative serum CA15-3 available for 1149 patients. Patients with elevated results showed a reduced 5 year disease-free survival (DFS) of 74.4% as compared to 84.7% (p=0.001). CA15-3 remained an independent prognostic factor in multivariate analysis (nodal status, grading, HR). Within HR positive disease, normal CA15-3 values predict a significantly better 5 year DFS of 89.9% compared to 79.7% (p=0.001). No significant difference was seen in HR negative patients (5 year DFS 57.5% vs 68.4%; p=n.s.). Patients with HER2 status available (n=700) were classified into biological tumor types by IHC. Significant differences in DFS were seen for “luminal” (n=435) tumors only (5 year DFS 81.0% vs 91.8% (p=0.001). Only trends of differences in DFS were seen in the other less frequent tumor types “luminalHER2” (n=90) or “triple neg.” (n=112) types. Within the “HER2” (n=63) group no discrimination by serum levels of CA15-3 was seen. CEA did not add information on prognosis.

Discussion: Decision on adjuvant therapy is increasingly based on tumor biology. Particularly, in “luminal” tumors additional prognostic information is needed to decide on adjuvant chemotherapy. CA15-3 may be an easily available, independent marker to identify patients with a higher risk of recurrence within this group. Prospective validation and comparison to molecular typing is needed.

P4-09-06
The Prognostic Value of Tumour-Stroma Ratio in Triple Negative Breast Cancer.
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Background: Triple-negative breast cancer constitutes one of the most challenging groups of breast cancer given its aggressive clinical behaviour, poor outcome and lack of targeted therapy. Until now, profiling techniques have not been able to distinguish between patients with good and poor outcome. Recent studies suggested an important role for stroma in tumour growth and progression. In colorectal-, oesophageal- and breast cancer, the tumour-stroma ratio was found to be of prognostic value.

Objective: to evaluate the prognostic value of the tumour-stroma ratio in triple-negative breast cancer.

Methods: During the period January 2004-2008, 124 consecutive triple negative breast cancer patients treated in our hospital were retrospectively evaluated. Routine Haematoxylin-Eosin (H&E) stained histological sections were evaluated by two investigators (kappa 0.735) for stroma percentage, growth pattern (pushing margin), necrosis and amount of lymphocytic infiltrate. Patients with less than 50% stroma were classified as stroma-low and patients with ≥50% stroma were classified as stroma-high.

Results: of 124 triple-negative breast cancer patients, 50 (40%) had a stroma-high and 74 (60%) had a stroma-low tumour. Survival analysis revealed a 5 years relapse free period (RFP) of 85% in the stroma-low and 45% in the stroma-high group. Overall survival (OS) was 89% for stroma-low and 65% in the patients with a stroma-high tumour. Both RFP and OS were significantly worse in patients with stroma-high tumours compared to stroma-low. In a multivariate cox-regression analysis, tumour stroma remained an independent prognostic variable for RFP (HR 2.39; 95% CI 1.07-5.29; p=0.033) and OS (HR 3.00; 95% CI 1.08-8.32; 0.034) when corrected for other clinical-pathological variables. In addition, tumour-stroma proved to be a strong prognostic variable when compared to nodal status, tumour grade and tumour size, with respectively a HR of 2.39 (95% CI 1.37-6.26) versus HR 2.18 (95% CI 1.06-4.48), HR 2.10 (95% CI 0.89-4.91) and HR 0.53 (95% CI 0.70-3.91) for RFP with comparable numbers for OS.

Conclusion: Tumour-stroma ratio is a strong independent prognostic variable in triple-negative breast cancer. It is easy to determine, reproducible (kappa 0.735) and can be easily incorporated into routine histological examination. This parameter optimizes risk stratification and could be target for future therapies.
P4-09-07

Breast Cancer Outcome by Combined Immunohistochemical ER/PR/HER2 Receptor Phenotype.


Introduction: We previously reported that molecular prognostic subgroup classification of operable breast cancers resembles the classification based on combined immunohistochemical expression of ER, PR and HER-2 receptors. This update represents a larger cohort with more detailed follow-up (median 5.5y).

Patients and methods: A prospective cohort of 4334 breast-cancer patients primary operated at our institution between 2000 and 2009. Steroid receptors were considered positive for any nuclear staining, HER-2 for strong (3+) membrane staining or positive fluorescence in situ hybridization. Survival curves were calculated for six predefined breast cancer subgroups: ER + PR + HER-2 - (PPN), ER + PR - HER-2 - (PNN), ER + PR + HER-2 + (PPP), ER - PR - HER-2 - (NNN), ER - PR - HER-2 + (NPP), and ER + PR + HER-2 + (PNP) (figures not allowed in abstract). Disease free interval (DFI), distant metastasis free interval (DMFI), overall survival (OS) and breast cancer specific survival (BCSS) were calculated. A long-rank test was used for testing the null hypothesis of no difference between the groups.

Results: We previously reported that molecular prognostic subgroup classification of operable breast cancers resembles the classification based on combined immunohistochemical expression of ER, PR and HER-2 receptors. This update represents a larger cohort with more detailed follow-up (median 5.5y).

Conclusion: Combined receptor expression for ER/PR/HER2 determines specific breast cancer subtypes with significant different clinical behavior. Both predictive and prognostic factors need to be considered when interpreting these results.

P4-09-08

Secretory Leukocyte Protease Inhibitor as a Diagnostic Differential Biomarker of Tumor Emboli in Inflammatory Breast Cancer.

Robertson FM, Ye Z, Chu K, Luo AZ, Wright MC, Jin J, Barsky SH, Cristofanilli M, Krishnamurthy S. The University of Texas MD Anderson Cancer Center, Houston, TX; University of Nevada School of Medicine, Reno, NV; Nevada Cancer Institute, Las Vegas, NV; Fox Chase Cancer Center, Philadelphia, PA

Background: Inflammatory breast cancer (IBC) is the most aggressive and lethal variant of locally advanced breast cancer. Pathologically, IBC is characterized by the presence of nests of cells, defined as tumor emboli, that undergo lymphovascular invasion into the skin and chest wall and represent the metastatic lesion of IBC. Due to the distinct presentation of IBC, it is often misdiagnosed due to a lack of defined biomarkers that are specific for tumor emboli in biopsy samples of IBC patients. To date, the only validated marker that has shown to consistently identify IBC tumor emboli is the surface glycoprotein E-cadherin.

Materials and Methods: With the goal of identifying genes specifically expressed by IBC tumor emboli, the present studies performed whole transcriptome analysis of tumor emboli from skin punch biopsy samples from IBC patients and tumor emboli from the Mary-X pre-clinical model of IBC which were isolated using laser capture microdissection.

Results: Secretory leukocyte peptidase inhibitor (SLPI) was a gene identified as being highly expressed by tumor emboli. This gene was previously identified as a metastasis related gene, has been reported to be associated with poor prognosis in serous ovarian carcinoma and is implicated in mediating resistance to paclitaxel. Immunofluorescence staining and confocal microscopy revealed that SLPI is a robust marker of tumor emboli in skin punch biopsy from IBC patient and in Mary-X tumor emboli. SLPI differentially delineates between IBC tumor emboli and hair follicles present in the skin, which also stain with E-cadherin. An additional marker, Cytokeratin 15 (CK15), can be used in combination with SLPI to differentially identify the components of the skin that are CK15 positive, and IBC tumor emboli that reside within the skin, which are CK15 negative. Use of podoplanin antibodies, which stain lymphovascular endothelium, demonstrate that IBC tumor emboli that express SLPI protein, are completely encircled within the dermal lymphovasculature.

Conclusions: These studies are among the first to identify SLPI as a potential biomarker of tumor emboli in skin punch biopsy samples from IBC patients and in pre-clinical models of IBC. Studies are ongoing to establish the molecular function and role of SLPI in mediating the survival and invasion of IBC tumor emboli. Collectively, these studies are the first to identify SLPI as a potential biomarker of IBC tumor emboli in skin punch biopsy samples. In addition to serving as a potential biomarker of tumor emboli, SLPI may also serve as a therapeutic target for development of approaches to eradicate IBC tumor emboli.

P4-09-09


Raghav KP, Wang W, Liu S, Chavez-MacGregor M, Menc X, Hortobagyi GN, Mills GB, Meric-Bernstam F, Blumenschein GR, Gonzalez-Angulo AM. MD Anderson Cancer Center, Houston, TX

Background: c-MET is a tyrosine-kinase membrane receptor and its dysregulation is involved in tumor proliferation, survival, angiogenesis, and migration. High levels of c-MET have been
observed in various tumor types and correlate with adverse outcome. The purpose of this study was to evaluate levels of total cMET and phospho-cMET (p-cMET) in breast cancer and their impact on survival outcomes.

**Materials and Methods:** We measured quantitative expression of cMET and p-cMET in a cohort of 257 breast cancer primary tumor samples using reverse phase protein array. The level of cMET/p-cMET in each sample was expressed as a log-mean centered value after correction for protein loading with the use of the average expression levels of > 50 proteins. The regression tree method and Mantingale residual plots were applied to find the best cutoff point for high and low levels of each protein. Linear regression models were used to determine if mean expression was different among breast cancer subtypes. The Kaplan-Meier method was used to estimate relapse-free survival (RFS) and overall survival (OS) by cMET and p-cMET levels. Cox proportional hazards models were fit to determine the association of cMET and p-cMET levels with the risk of recurrence and death after adjustment for other patient and disease characteristics.

**Results:** Median age was 51, (range 23-85) years. There were 140 (54.5%) hormone receptor (ER/PR)-positive, 53 (20.6%) HER2-positive, and 64 (24.9%) triple-negative tumors. There were no significant differences in the mean expression of cMET (P=0.128) and p-cMET (P=0.088) by breast cancer subtype. Dichotomized cMET and p-cMET expression was a significant prognostic factor of RFS (HR: 0.41, 95% CI: 0.23-0.75, P=0.004, and HR: 0.61, 95% CI:0.38-0.96, P=0.033, respectively) and OS (HR: 0.31, 95% CI:0.14-0.70, P=0.005, and HR: 0.52, 95% CI:0.29-0.93, P=0.025, respectively). Within breast cancer subtypes, high cMET expression was associated with worse RFS (P=0.02) and OS (P=0.01) in ER/PR-positive tumors, and high p-cMET expression was associated with worse RFS (P=0.03) and OS (P=0.03) for patients with HER2-positive breast cancer. Multivariable model after adjustment for patient and tumor characteristics showed that patients with tumors with high cMET levels had a significant higher risk of recurrence (HR: 0.28; 95% CI, 0.36-0.80) and death (HR: 0.24; 95% CI, 0.09-0.65). Similarly, patients with tumors with high p-cMET levels had a significant higher risk of recurrence (HR: 0.53; 95% CI, 0.29-0.97).

**Conclusion:** In this cohort of patients, high expression of cMET and p-cMET was seen in all subtypes of breast cancer. High levels of cMET and p-cMET had a significant impact on breast cancers survival outcomes. cMET inhibition may a be promising novel target for therapy in breast cancer and deserves investigation.

**P4-09-10**

**Assessment of Circulating Immune Parameters in Patients with Metastatic Breast Cancer Improves Survival Prediction.**


**Background:** Prediction of survival for metastatic breast cancer (MBC) patients (pts) remains a major clinical challenge. Most studies proposed prognostic scores based on clinical criteria that are often subjective. We already demonstrated that low lymphocyte count is a prognostic factor in various pts populations, and we recently showed that low CD4 lymphocyte count is a prognostic factor for overall survival (OS) in MBC pts. In this study, we evaluate the plasma levels of various cytokines and chemokines to improve our immune-based prognostic model.

**Methods:** The first cohort (A) consisted of 39 pts with MBC treated with first line chemotherapy between Sep. 04 and Oct. 07. The second cohort (B) consisted of 114 pts with MBC who relapsed after at least one line of chemotherapy between Dec. 2000 and Nov. 05 and who received further chemotherapy. In the cohort A the blood samples were drawn before administration of any chemotherapy. In the cohort B the samples were drawn after white blood cell recovery. Fresh cells have been used for extensive phenotypic analyses. Plasma cytokines levels have been measured using commercially available Lumineux-based multiplex kits. Cytokines were clustered into 3 groups based on biological pathways, group 1: Th1 response and T-cell proliferation (IL-2, IL-7, IL-15, IFNγ, IL-12p40, IFNα2, GM-CSF); group 2: Th2 response (IL-10, IL-13, CCL22), and group 3: inflammatory response (IL-1b, IL6, IL-17, TNFα).

**Results:** In the cohort A: CD4+ T cell levels <450/µL were associated with worse OS (HR=2.46 [95%CI=1.21-5.01], p=0.013). In the cohort B, 48% of pts had received one previous line of chemotherapy; 52% had received more than one. CD4+ T cell levels <450/µL were also associated with worse OS (HR=1.70 [95%CI=1.04-2.78], p=0.036). Analyzing cytokines by clusters, elevation in group 1 (Th1 response) was associated with poor OS for both cohorts (A=HR=1.055 [95%CI=1.002-1.111], p=0.041) and B (HR=1.12 [95%CI=1.01-1.24], p=0.032). Conversely, the group 2 (Th2 response) was not associated with OS for both cohorts. For the group 3 (inflammatory response), the increase of these cytokines values was a prognostic factor of OS only for the cohort B (HR=1.10 [95%CI=1.02-1.18], p=0.009). In multivariate analysis: in the cohort A, CD4+ T cell levels <450/µL (HR=2.45 [95%CI=1.20-5.03], p=0.014) and group 1 of cytokines (HR=1.055 [95%CI=1.004-1.109], p=0.034) remains independent prognostic factors; in the cohort B, poor Performance Status (HR=3.10 [95%CI=1.99-4.86], p<0.0001) and group 3 of cytokines (HR=1.09 [95%CI=1.01-1.17], p=0.020) were shown to be independent prognostic factors.

**Conclusions:** Combination of PS and biological covariates such as lymphocyte CD4+ count or cluster of cytokines is an effective strategy to predict survival of pts with MBC receiving first-line chemotherapy or subsequent lines. The validation of our immune-based prognostic score (combining cytokine levels and immune cell phenotypes) will be initiated in a prospective study.

**P4-09-11**

**Kinesin Family Member 2C (KIF2C) Is a New Surrogate Prognostic Marker in Breast Cancer (BC).**

*Abdel-Fatah TMA, Green AR, Lemenet C, Moseley P, Chan S, Ellis IO, Balls G. Nottingham University City Hospital NHS Trust, Nottingham, United Kingdom; Nottingham University, Nottingham, United Kingdom; Nottingham Trent University, Nottingham, United Kingdom*

**Introduction**

Gene expression microarrays, artificial neural network (ANN), tissue microarray and immunohistochemistry (IHC) techniques allow for the analysis of huge numbers of gene transcripts and their corresponding proteins and have been widely applied in predicting clinical outcome.

**Methods**

1. In this study, we analysed 48,000 gene transcripts of 171 unselected series of BC using ANN and pathways analysis to identify genes that can be used to predict clinical outcome of BC.

2. The clinic-pathological outcome of candidate genes were validated by using IHC in 4 independent primary BC data sets: a) a series of 379 consecutive high risk BC (NPI>3.4) who treated with surgery (S)+ radiotherapy (RT) and did not received neither endocrine (ET) nor chemo-therapies (CT), b) A series of 1650 consecutive cases of
BC who treated with S + RT and received adjuvant CMF and/or ET according to Nottingham prognostic index (NPI), menopausal and ER status, c) 250 locally advanced BC treated with anthracycline-based combination with or without Taxane followed by S + RT and d) 145 BC overexpressing HER-2 treated with S + RT followed by sequential adjuvant anthracine combination CT + trastuzumab.

**Results**

**Gene expression analysis**

ANN analysis revealed that KIF2C gene was the highest ranked gene that predicted clinical outcome and accurately differentiated between low and high grade BC based on a 10-fold external cross-validation analysis with an average classification accuracy of >98%. High KIF2C gene expression level was associated with shortest BC specific survival (BCSS), disease free (DFS) and distant metastasis free survivals (DM-FS); p<0.0001. In univariate analysis, high level of KIF2C gene expression was associated with large tumour size, higher lymph node stage, negative ER, positive p53 expression and HER2 overexpression. However in multivariate analysis, KIF2C gene expression level was only statistically associated with histological grade (p=0.0001) and mitosis (p<0.0001). Pathways analysis revealed that KIF2C is likely to play a significant role in cytokinesis, cell division and cell cycle regulations.

**Immunohistochemistry**

75% of BC showed overexpression of KIF2C protein. KIF2C protein overexpression was associated with unfavourable clinicopathological features including high grade, high mitotic index, basal like phenotype, triple negative phenotype, HER2 overexpression, TOP2A overexpression, p53 mutation, and loss of BRCA1 (adjusted p=0.0001).

In univariate analysis, KIF2C protein overexpression was associated with patient’s BCSS in both ER+/high and ER/-high risk patients (NPI > 3.4) who did not received ET (HR: 3.3, 95% CI: 1.2-9.3, p=0.02) and ER-/ high risk patients who did not received CT (HR: 3.2, 95% CI: 1.1-8.8, p=0.025).

In 1650 BC series, multivariate Cox regression model including validated prognostic factors, confirmed that KIF2C overexpression is an independent prognostic factor. KIF2C overexpression showed increase in the risk of death (HR: 1.5, 95% CI: 1.1-2.0, p=0.009), recurrence (HR: 1.4, 95% CI: 1.1-1.8, p=0.017) and DM (HR: 1.6, 95% CI: 1.2-2.3, p=0.005).

In conclusion, our findings provide a new insight to a better understanding of mammary carcinogenesis and that KIF2C is a promising molecular prognostic factor and a potential therapeutic target.

**P4-09-12**

**Clinical Validation of Immunohistochemical Signature Predictive of Patients’ 8 Year Outcome in Node Negative Breast Carcinomas**

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**Background:** Immunohistochemical (IHC) signatures predictive of prognosis can be a cost effective alternative to genomic signature suitable in clinical practice for patients' management in early stages of breast carcinoma (BrCa) in clinical. IHC procedures are very easy to handle in pathology laboratories, since require very few amount of formalin fixed tissue samples since assessed on consecutive 4 µm thick sections of paraffin embedded tumors fragments validated by pathologists’ expertise. We recently reported (C Charpin et al Int J Cancer 2009) that standardized IHC assays using high throughput densitometry on digitized microscopic images allow to predict an adverse outcome of patients with BrCa, provided that identified the IHC predictive signatures can be validated in other patients’ cohorts. In this regard we have repeated our previously reported IHC procedures on 418 series of BrCa (Marseille University) on a second series including 303 BrCa diagnosed at Yale University.

**Material and Methods:** TMA(s) of two cohorts of patients with BrCa (418/Marseille University and 303 Yale University) were respectively investigated for immunohistochemical expression of 15 markers, including (i) those of the best signature predictive of patients outcome in N- patients in our previous studies and (ii) new markers reported in the literature as EMT cells and Stem cells. Monoclonal antibodies against HIFa, PI3, pAKT, pmTOR, moesin, P21, 4E-BP, p70S6K, Ker 5.6, pMAPKAPK-2, SHARP2, Claudin, ALDH, AF6, CD24, IHC was screened using standardized (Ventana Benchmark XT) on consecutive 4 µm thick sections of TMA(s) from both series. Quantitative measurements of immunoprecipitates were automatically assessed with TRIBVN device, and correlated to 8 year patients’ outcome. Logistic regression determined the best combination of markers to predict patients’ prognosis and results of the two cohorts were compared.

**Results:** In node negative BrCa the best predictive combination of markers signature was HIF-1a, PI3K, Claudin, AF6, pAKT independently of ER PR and HER-2 status. When results of logistic regression with these markers was compared very similar results were obtained with 92.34% (333/418) of well classified patients in the first set (Marseille University series) and 89.8% (158/176) in the second set from Yale University.

**Discussion:** The results suggest that the new optimal IHC signature predictive of prognosis identified in BrCa including HIF-1a, PI3K, Claudin, AF6 and pAKT is validated by this study with very similar results in two different cohorts of patients. This validation also suggests (1) that IHC signatures investigated in individual patients can be considered as suitable in clinical practice offering a cost effective convenient mean to predict patients’ outcome at diagnostic time (2) and to select those patients with poor prognosis, particularly in N- BrCa subset, requiring more aggressive adjuvant therapies, and to avoid useless expensive therapies and their side effects in N- patients with favourable prognosis.

**P4-09-13**

**External Validation of Adjuvant! Online Breast Cancer Prognosis Tool. Improvement Is Still Needed.**


**Introduction:** AdjuvantOnline is a web-based application designed to provide 10 years survival probability patients with breast cancer. Few validation studies have underlined some limitations, particularly an overestimation of the prognosis among certain subgroups of patients. Moreover, several predictors such as HER2 over expression status and proliferation markers have not been assessed in Adjuvant! original study. We provide the validation of AdjuvantOnline algorithm on two breast cancer datasets collected from two large European cancer centres, and we determined whether the accuracy of AdjuvantOnline is improved by others well known prognostic factors.

**Material and Methods:** The French data set is composed of 456 women with early breast cancer, treated at the Institut Curie between 1995 and 1996. The dutch data set is composed of 295 women less than 50 years treated at the Netherlands Cancer Institute between 1984
and 1995. Agreement between observation and Adjuvant! prediction was checked by testing that the calibration slope was equal to 1. Logistic models were performed to evaluate whether risk factors adds significant prognostic information, including AdjuvantOnline a priori information as an offset.

**Results:** Ten years survival status was known for 383 patients in the French data set and 247 patients in the Dutch data set. Adjuvant! prediction was globally well calibrated in the French data set (observed survival 86%, predicted survival 85%), but was overestimated in high grade, HER2 positive and Ki67 > 20% subgroups. HER2 status, Mitotic Index, Ki67 and treatment type were strongly associated with 10-year survival, even considering AdjuvantOnline a priori information. In the Dutch data set, the overall 10-year survival was underestimated by AdjuvantOnline (observed 66%, predicted 79%), particularly in patients less than 40 years old.

**Conclusion:** AdjuvantOnline needs to be updated to adjust overoptimistic results in young and high grade patients, and should consider candidates, such as Ki67, HER2 and Mitotic Index.

### P4-09-14

**Differential Expression of the Akt1 Isoform in Mammary Ductal Carcinoma.**

**Sanders AJ, Mansel RE, Jiang WG. Cardiff University School of Medicine, Cardiff, United Kingdom**

**Introduction:**

AKT1 is an oncogenic protein and controls numerous cellular processes in both normal and cancerous cells. There are three isoforms, Akt1 (PKBα), Akt2 (PKBβ) and Akt3 (PKBγ). Frequently, regulation of the Akt pathway is disrupted in cancerous cells and interest within the scientific community has focused on the use of this pathway in treating cancer. However, recent studies have also implicated that Akt1 may act to limit invasive migration of breast cancer cells suggesting the potential of this isoform to have both pro- and anti-tumorigenic roles. In the current study we examined the expression profile of Akt1 in a cohort of ductal carcinoma of the breast.

**Methods:**

Quantitative transcript analysis was used to assess the expression levels of Akt1 in a range of ductal carcinoma of the breast (n=85) and normal samples. Expression levels were used in conjunction with clinical and pathological data, as well as the clinical outcomes of the patients (median follow-up 120 months).

**Results:**

Expression of Akt1 levels in the mammary ductal carcinoma cohort demonstrated a lower level of transcript expression in the lower grade and stage cancers. Levels of Akt1 were found to be significantly lower in grade 1 ductal breast carcinomas compared to the combined higher grade 1 and 2 carcinomas (ductal carcinoma grade 1 median = 3.7 vs ductal carcinoma grade 2,3 median = 33.6, p = 0.01). In addition to this, median transcript levels of Akt1 were found to increase with increasing TNM stage of the ductal carcinoma (Median values of Akt1 in ductal carcinoma TNM 1 = 5.9, TNM 2 = 63.6, TNM 3 = 533 and TNM 4 = 13680). This enhanced expression of Akt1 in higher TNM stages, compared to TNM stage 1, was found to be significant in most cases (TNM1 vs TNM2, p = 0.03; TNM1 vs TNM3, p = 0.15; TNM1 vs TNM4, p = 0.03).

**Conclusion:**

Variable expression of Akt1 was seen throughout the mammary ductal carcinoma cohort and correlated with a number of prognostic factors. Akt1 transcript levels were found to be significantly reduced in the low grade 1 and TNM1 stages when compared to the higher grades and TNM stages. Our current data suggests that low levels of Akt1 may be associated with better clinical patient prognosis in mammary ductal carcinoma.

### P4-09-15

**Clinical and Pathological Predictors of Outcome among Patients with Triple Negative Breast Cancer.**

**Pearlstone DB, Gray M, Garofalo RM, Trapani M, Nyirenda T, Hazelwood VA. Hackensack University Medical Center, Hackensack, NJ; Stevens Institute of Technology, Hoboken, NJ; John Theurer Cancer Center, Hackensack, NJ**

**Background:** Triple negative (TN) breast cancer portends a poor prognosis, yet outcomes remain heterogeneous. Specific characteristics of individual TN patients that can predict outcome compared to non-triple negative (NTN) patients have yet to be well defined.

**Methods:** A prospective oncology database at Hackensack University Medical Center was queried for all patients with invasive breast cancer between 2000 and 2005. Patients with triple negative breast cancer were identified and compared to non-triple negative patients. Numerical values were compared by Mann-Whitney test; categorical values by Chi square/Fisher exact test. Log-rank test was used to compare outcome statistics, univariate and multivariate analyses were used to identify factors affecting relapse-free survival and overall survival. **Results:** A total of 2,216 patients were identified (TN: n=260; NTN: n=1956). There was no difference in race (white vs non-white; TN: 82.3% vs NTN: 87.3%) or family history of breast cancer (TN: 19.2% vs NTN: 16.8%). TN patients were significantly younger (median age; TN: 55.5 vs NTN 57.7; p < .05), had larger tumors (mean cm; TN: 3.1 vs NTN: 2.4; p < .05), had higher grade tumors (% grade 3; TN: 74.6 vs NTN: 26.1; p < .0001), had higher proliferation index (>20% nuclei stained for Ki-67; TN: 30.8% vs NTN: 6.7%; p < .0001), but were less likely to have lymph node metastases at presentation (TN: 35.7% vs NTN: 48.0%; p < .0001). Among node negative patients, overall survival (OS) and disease free survival (DFS) were not significantly different between TN and NTN. Among node positive patients, both OS and DFS were worse for TN patients compared to NTN patients (p < .0008).

**Conclusion:** Although TN patients have poor outcomes overall, among node negative patients, TN status does not affect overall nor disease free survival. Among node positive patients, however, TN status, nodal status and tumor size were all independently associated with DFS.

### P4-09-16

**Factors Affecting the Development of Axillary Lymph Node Metastases in T1a-T1b Breast Cancers.**

**Khair TA, Boolbol SK, Boachi-Adjei K, Klein P. Beth Israel Medical Center, Continuum Cancer Centers of New York, New York, NY**

**Background:** The presence of axillary nodal metastases is a poor prognostic indicator in patients with breast cancer. In the majority of patients, axillary nodal involvement correlates with tumor size and most commonly in tumors greater than 1 cm. Many series report an incidence of axillary nodal metastases in 3-37% of patients with tumors less than or equal to 2 cm. The presence of axillary nodal metastases will often change the stage of the cancer and role of it.
adjuvant systemic treatment; therefore, it is important to understand what features are associated with these small lymph node positive tumors. The purpose of this study is to identify characteristics of T1a and T1b breast cancers that present with axillary nodal metastasis.

Methods: A retrospective review of a prospective database identified 878 patients with T1a and T1b breast cancer. Pathologic features including tumor grade, hormone receptor status, lymphatic and vascular invasion, Her-2 neu status, and tumor histology were examined in patients with and without axillary nodal metastases. Pearson’s χ² test along with stepwise linear regression analysis were used to determine which factors were associated with the development of axillary nodal metastases.

Results: There were 289 T1a lesions and 589 T1b lesions studied. Four independent variables were found to be associated with axillary nodal metastases in T1a-T1b breast cancer. As illustrated in Table 1, a significant difference was seen in nodal positivity among patients with ER negative tumors compared to those with ER positive tumors (p = .0000). An increased incidence of nodal positivity was also observed among patients with LVI compared to those without LVI (p = .0000). A significant difference was observed in nodal positivity among patients with perineural invasion compared to those without (p = .0006). An increased incidence of nodal positivity was also observed among T1a and T1b tumors with a higher histologic grade (p = .0409, p = .0001). Of note, when comparing T1a to T1b breast cancers, there was no significant difference found among the identified exposure variables of interest.

Table 1.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Node Positive Odds Ratio</th>
<th>p value</th>
<th>Node Negative Odds Ratio</th>
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<td>[CI 1.01, 5.47]</td>
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</table>

Conclusion: In this series, the overall prevalence of nodal positivity in T1a and T1b breast cancer is 12%. An absence of a relationship between T1a and T1b cancers with nodal positivity was observed. However, the presence of nodal positivity showed a significant correlation with ER negativity, LVI, perineural invasion, and higher histologic grades.

P4-09-17

Leptin and Adiponectin Expression in Breast Cancer.

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Background: Several adipocytokines, such as leptin, resistin, and adiponectin, are associated with obesity and breast cancer. Multiple studies have indicated that adipocytokines may significantly influence the growth and proliferation of tumors. However, the relationship between adipocytokines and breast cancer invasiveness has not been investigated in human tissue. The aims of this study were to determine the expression of leptin, leptin receptor (ObR), adiponectin and adiponectin receptor (AdipoR) in human breast cancer, to evaluate their prognostic significance in the breast cancer.

Material and Methods: Specimens from 198 patients with primary breast cancer were enrolled, and representative paraffin tumor blocks were selected for constructing tissue microarrays (TMA). Immunohistochemical staining for leptin, ObR, adiponectin, and AdipoR was performed using TMA, and the clinicopathologic characteristics were evaluated from the patient’s medical records.

Results: Stage 0 breast cancer accounted for 41 cases, and 157 cases were invasive cancer. A univariate analysis showed that positive rates of leptin and ObR expression in the ductal carcinoma in situ (DCIS) group were significantly higher than those of the invasive cancer group (97.4% vs. 34.0%, p = 0.001; 74.4% vs. 29.8%, p = 0.001). However, positive rates of adiponectin and AdipoR expression in the invasive cancer group were significantly higher than those in the DCIS group (53.7% vs. 33.3%, p = 0.024; 59.9% vs. 26.3%, p = 0.001). High leptin expression was significantly associated with high Ki-67 expression (p = 0.016). High adiponectin expression was significantly correlated with smaller tumor size (p = 0.001). The disease-free survival (DFS) and overall survival (OS) rate at 5 years were 89.3% and 96.7%, respectively. No difference in DFS and OS was observed based on leptin, ObR, adiponectin, AdipoR expression.

Discussion: Previous studies, using serum of breast cancer patients or breast cancer cell line, revealed that leptin and ObR may affect processes related to cancer initiation and progression, and adiponectin has protective effect on tumor progression. However, the precise effects of leptin, ObR, adiponectin and AdipoR in breast cancer are still unclear. Our results suggest that losses of leptin and ObR expression could be associated with invasive cancer, whereas high adiponectin and AdipoR expression may be associated with breast cancer invasiveness.

P4-09-18

Australian Decision Impact Study: The Impact of Oncotype DX Recurrence Score (RS) on Adjuvant Treatment Decisions in Hormone Receptor Positive (HR+), Node Negative (N0) and Node Positive (N+) Early Stage Breast Cancer (ESBC) in the Multidisciplinary Clinic (MDC).

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Introduction: There has been increasing interest in more accurately defining the need for chemotherapy in patients with HR+ ESBC. Oncotype DX® has been shown to provide prognostic and predictive information beyond traditional histopathological factors. Prior clinical impact studies have focused on decisions made by individual practitioners. In Australia, the MDC has become the standard forum for determining treatment decisions. In this study, we have assessed how the RS influences adjuvant systemic treatment decisions in the MDC at 4 separate hospitals. Methods: Patients with unifocal HR+, HER2-ve ESBC, N0 and N+ (1-3 positive nodes, including micrometastases and isolated tumour cells) and no contraindication to adjuvant chemotherapy (CTx) or endocrine therapy (H) were eligible. A total of 150 patients will be entered. Systemic adjuvant treatment recommendations were made and recorded in the MDC, both before and after testing with Oncotype DX. The primary endpoint was the overall change in the treatment recommendation. Other endpoints included confidence in recommendations, assessment of impact of the RS, and treatment the patient actually received. Results: Currently, RS results on 84 patients are available. Patient age range is 33-82 years. There are 56 (67%) N0 pts and 28 (33%) N+. Overall, 37 (44%) women had low, 36 (43%) intermediate and 11 (13%) high RS. The RS changed the initial treatment recommendations in 20 (24%) patients. Of the 34 patients initially recommended CTx+H, there was a shift to H alone for 12 (35%), whilst of the 50 patients initially recommended H alone, 7 (16%) shifted to CTx+H. A shift in treatment recommendations was reported for 8/37 (22%) pts with low, 8/36 (22%) with intermediate and 4/11 (36%) with high RS.
13/56 (23%) of N0 pts had a change in recommendation, whilst 7/28 (25%) N+ pts changed (table 1). 5 patients have decided against the final MDC recommendations: 3 chose CTx+H after recommendation of H (RS of 13, 19 and 21 respectively), and 2 declined a CTx+H recommendation (RS of 19 and 25). Conclusion: Early results of our Australian study suggest an impact of Oncotype DX RS on adjuvant treatment decision making in the MDC setting. Updated results on the full 150 patients will be presented at the meeting.

Table 1

<table>
<thead>
<tr>
<th>Nodal Status</th>
<th>N0 (n=56)</th>
<th>N1-3 (n=28)</th>
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</thead>
<tbody>
<tr>
<td>Post-RS Rec.</td>
<td>CTx + H</td>
<td>H alone</td>
</tr>
<tr>
<td>CTx + H</td>
<td>8</td>
<td>13</td>
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<tr>
<td>H alone</td>
<td>8</td>
<td>15</td>
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P4-09-19

PCNA+ Tumor Associated Macrophages Are Associated With M1 and M2 Gene Expression, and Confer Poor Prognosis in the Absence of Anti-Tumor Immune Environment.


Background

Tumor associated macrophages (TAMs) promote breast tumor progression through the production of angiogenic factors, stromal breakdown factors, and the suppression of adaptive immunity. TAMs are recruited from the circulation to the tumor site, and can undergo a spectrum of phenotypic changes, with two contrasting activation states described in the literature: the M1 anti-tumoral and M2 pro-tumoral phenotypes. We previously identified a population of PCNA+ TAMs associated with high grade, hormone receptor (HR) negative tumors with poor outcomes. We hypothesized that high PCNA+ TAMs would be associated with expression of M2 related genes.

Methods

We used immunohistochemistry to measure PCNA+ TAM levels (double positive for PCNA and CD68) in 135 invasive breast cancer cases from the I-SPY 1 Trial, a prospective neoadjuvant trial with serial core biopsies and gene array data. We developed gene-sets representing M1 related, M2 related, and anti-tumor immune response (represented by cytotoxic T cells and MHC Class II) genes based on literature review. We compared PCNA+ TAM levels, expression of these gene-sets, and outcomes.

Results

Higher than mean PCNA+ TAM counts were associated with increasing grade (p < 0.001), HR negativity (p < 0.001), and decreased recurrence free survival (RFS, p = 0.05). Among subjects who had a pathologic complete response (pCR), there was no difference in RFS between those with high versus low PCNA+ TAMs. Among subjects without pCR, those with high PCNA+ TAMs had significantly worse RFS than those with low PCNA+ TAMs (p = 0.0028). In the 95 subjects with both PCNA+ TAM results and gene expression arrays available, high PCNA+ TAM levels were associated with more M1 than M2 related genes. The gene-set representing anti-tumoral immune environment was not by itself associated with RFS. However, those subjects with both high PCNA+ TAMs and the absence of anti-tumoral immune response gene expression had significantly worse RFS than those with high PCNA+ TAMs but the presence of anti-tumoral immune related genes (p = 0.01).

Conclusions

High PCNA+ TAMs had different effects on outcomes depending on tumoral immune environment. Instead of being purely M2 macrophages, PCNA+ TAMs likely represent a heterogeneous mixture of TAMs with different polarization states. Additional markers are needed to distinguish anti-tumoral from pro-tumoral PCNA+ TAMs.

P4-09-20

Expression Profile of Interleukin 17B and the Receptor IL-17BR in Clinical Breast Cancer.

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Introduction:

Interleukin 17B (IL-17B) is a member of the interleukin 17 cytokine family. Members of this family have been implicated in conditions such as autoimmune diseases and the inflammatory response. A number of studies have examined the role of IL17A in cancer progression and angiogenesis and shown a variety of functions, both pro- and anti-tumorigenic. Currently there is little data on IL-17B and the receptor IL-17BR and their involvement in cancer progression. The current study looks at the expression profile of IL-17B and IL-17BR in a clinical breast cancer cohort.

Methods:

The expression profile of IL-17B and IL-17BR was examined in a cohort of human normal breast and breast cancer specimens (normal, n = 34; tumour, n = 109). IL-17B and IL-17BR transcript expression in the samples was analysed using Q-PCR and compared to clinical and pathological data.

Results:

Both IL-17B and IL-17BR expression seemed to correlate with NPI staging. IL-17B levels were low in patients with NPI-1 or -2 cancers (median values 3.1 and 1.1 respectively), with highest levels of IL-17B being observed in the poorer prognosis NPI-3 group (median value 206.9; p = 0.07 NPI-1 vs NPI-3 and p = 0.01 NPI-3 vs NPI-2). Similar to this IL17B levels in TNM stage 1 cancers were significantly lower than those in higher TNM stages (TNM1 median value = 0.3 vs grouped TNM234 median value 10.3, p = 0.03). IL-17BR expression also tended to increase with NPI staging (IL-17BR median expression, NPI-1 = 0.0, NPI-2 = 0.04 and NPI-3 = 1.59; NPI-1 vs NPI-3 p = 0.07). Additionally, levels of IL-17BR were found to be significantly lower in background tissue compared to cancer tissue (median values 0.00 vs 0.01 respectively, p = 0.014).

Conclusion:

IL-17BR expression seems to be higher in tumour tissue compared to normal background tissue. Additionally, expression of IL-17B and IL-17BR both appear to be associated with higher NPI stage, particularly in the poor prognostic NPI-3 group. Expression of IL-17B seems to be elevated collectively in TNM stage 2, 3 and 4 compared to the better prognostic TNM1 stage. Together the data suggests that IL-17B and IL-17BR expression may be useful molecules to identify poorer prognostic breast cancers in patients.

P4-09-21

A Novel Prognostic Marker for Triple-Negative Breast Cancers.

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BACKGROUND: Triple-negative breast cancers (TNBC) represent a highly aggressive form of this disease with few treatment options.
available. Currently, even the smallest node negative cancers are considered by many to warrant treatment with chemotherapy (CTX). While many recur early (within 2-3 years), there is a subset of long-term survivors illustrating the heterogeneity within this group. Here we report our ongoing effort to establish tissue inhibitor of metalloproteinase-4 (TIMP-4) as a prognostic marker in all early breast cancers a. While the canonical function of TIMPs is to inhibit tissue degradation, numerous reports have established that TIMPs exert tumor promoting activity. In our prospective study, we evaluated TIMP-4 as prognostic marker for TNBC and its role in disease progression.

METHODS: Specimens from our retrospective and prospective cohorts were assessed by immunohistochemical staining using standard techniques and a monoclonal antibody for TIMP-4b in accordance with the IRB approved protocol. Staining intensity was documented on a scale of 0-3. No data was released to the treating physicians at the time of collection. Outcome data from a total of 240 pts was obtained through tumor registry and clinician practices. Staining intensity was then correlated to outcome to calculate sensitivity and specificity of the marker. To determine the role of TIMP-4 in TNBC cell behavior we have performed microarray analyses. The effects of TIMP-4-induced signaling were tested using invasion and clonogenic survival assays under normal growth conditions and after exposure to gamma radiation.

RESULTS: Elevated TIMP-4 expression identified a high risk of relapse and short survival time with 75% sensitivity and 80% specificity. No discernable differences were noted between retrospective and prospective cohorts. Array analyses revealed activation of the PI3K/AKT pathway in the presence of TIMP-4. Furthermore, elevated TIMP-4 increased the invasive behavior of breast cancer cells in Matrigel™-coated invasion chambers and reduced sensitivity to gamma irradiation. These effects were reversible by addition of either a PI3K inhibitor or an anti-TIMP-4 antibody, suggesting their use as potential therapeutic strategies.

CONCLUSIONS: On the basis of these clinical data we suggest that TIMP-4 may offer a simple prognostic marker for TNBC patients at highest risk. The presence of TIMP-4 identifies a patient population likely to recur quickly despite standard CTX treatment. Our research suggests that targeted therapy of the PI3K/AKT pathway and/or a biological therapeutic approach directed against TIMP-4 may be of benefit in this subset of pts and should be explored. Therefore, TIMP-4 testing of TNBC patients could aid in the selection of a treatment regimen to improve survival outcome.

P4-09-22

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Introduction

The oncotype dx (ODX) 21 gene recurrence score (RS), was intended to predict the 10 year risk of breast cancer recurrence for estrogen receptor positive, lymph node negative disease (NEJM 2004;351:2817-26). The proportions of patients that were reported as low, intermediate and high risk were 51%/22%, 27%. This population of patients was dominated by ductal carcinomas (DCA), with a paucity of classical lobular carcinomas (CLCA), which are ER-rich and low grade histology. To date, there is dearth of data regarding the relationship of the RS and CLCA, and the clinical relevance regarding the ODX RS in CLCA has not been studied.

Methods

As part of quality assurance review at three institutions, the ODX RS for all CLCA were reviewed in order to determine the perceived clinical relevance of the ODX RS compared to the proportions of RS reported (NEJM 2004;351:2817-26).

RESULTS

A total of 219 cases of ODX were performed on CLCA. The low/intermediate/high risk profile was 58%/40%/2%. Conclusions (1) The risk profile of ODX for CLCA is very different from the original DCA dominant population previously reported. (2) If the goal of ODX is to identify candidates for chemotherapy, then a high RS is a rare event for CLCA, and as such, the judgment to administer chemotherapy to an individual patient would most likely depend upon factors other than the ODX, because 98 percent of patients are combined low/intermediate risk RS.

P4-09-23
Monitoring Autophosphorylation of Mammalian Target of Rapamycin (mTOR) for Histoprognostic Grading of Invasive Breast Cancer: Impact on the Reclassification of Patients with Grade 2 Tumors Using the Nottingham Grading System.

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Histological grade is one of the strongest prognostic factors in operable breast carcinomas (BC). Although Grade 1 and Grade 3 are biologically and clinically distinct tumor entities that clearly separate low-risk from high-risk BC patients, 30-60% of BC are classified as histological Grade 2, which is associated with an intermediate risk of recurrence and is not informative for clinical decision making. Previous studies using a 97-gene measure of histological tumor grade (i.e. the Genomic Grade Index, GGI) have confirmed GGI’s ability to accurately reclassify Grade 2 patients into 2 BC groups with high versus low risks of recurrence. However, GGI’s applicability is still expensive, technically demanding and is not readily available in a routine clinical setting in most hospital laboratories. The prognostic abilities of BC gene expression signatures such as GGI are due mostly to the detection of proliferation activity. One of the strongest, yet simple and well-reproducible proliferation-associated prognostic factors is the mitotic activity index (MAI). Here, we have tested whether immunohistochemical assessment of MAI by assessing the autophosphorylation status of mammalian Target Of Rapamycin (mTOR) at Serine 2481 could significantly impact on the histopathological classification of Grade 2 BC when using the Nottingham Grading System. 1.) We validated the sensitivity of phospho-mTORSer2481 (PP-mTOR) labeling in detecting & counting mitotic figures and also its usefulness for histoprognostic grading in a series of 144 BC biopsies; 2.) We investigated the correlation between PP-mTOR MAI and the MAI determined by using an antibody selective for the Ser10-phosphorylated Histone H3 (PP-H3), a well-recognized mitosis-specific marker. PP-mTOR-labeled mitotic figures were easily seen and permitted a quick identification of the area of highest mitotic activity, even at low-power magnification. Our study showed a statistically significant correlation between the subjective mitotic counts obtained by using PP-mTOR labeling and those assessed by either standard H&E (r = 0.662) or staining with PP-H3 (r = 0.896). PP-mTOR MAI correlated also with tumor
Correlation of Aurora Family Member Expression with Clinical Breast Cancer Prognosis.

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Introduction:
Aurora-A, Aurora-B and Aurora-C are a family of protein kinases which have been identified as key regulators of the mitotic cell division process. Members of this group have been linked with pro-tumorigenic effects and have been reported as being up-regulated in certain cancers. Our current study examines the expression profile of the Aurora-A, Aurora-B and Aurora-C members in a breast cancer cohort in relation to a number of predictive factors.

Materials and Methods:
The expression of Aurora-A, Aurora-B and Aurora-C was examined in a breast cancer cohort using quantitative real time PCR (normal samples n = 34, tumour samples n = 109) and immunohistochemistry (IHC). Expression of the aurora members was related to clinical parameters such as staging and Nottingham prognostic index (NPI).

Results:
Examination of Aurora-A levels in the breast cancer cohort revealed an association of Aurora-A expression with NPI grouping. High levels of Aurora-A levels were seen in NPI-1 and NPI-2 stages (NPI-1 median = 66.0, NPI-2 median = 64.3), with expression reducing significantly at the higher NPI-3 group (NPI-3 median = 20.3; p = 0.029 vs NPI-2 and p = 0.009 vs NPI-1 respectively). Additionally, Aurora-A levels were found to be significantly lower in grade 3 cancers compared to grade 1 (median grade 1 = 86.5 vs median grade 3 levels = 52.6, p = 0.04). In contrast to this, examination of Aurora-C levels in the cohort identified a relatively low level of Aurora-C in NPI-1 and NPI-2 group (median values NPI-1 = 88, NPI-2 = 34.4) compared to a substantial increase in Aurora-C expression in NPI-3 (median value = 1602, p = 0.05 vs NPI-1 and p = 0.06 vs NPI-2). Aurora-B expression levels did not exhibit any significant differences within the breast cancer cohort.

Conclusions:
Differential expression of Aurora-A, Aurora-B and Aurora-C were observed within the breast cancer cohort. Higher Aurora-A expression was associated with low NPI groups and grades and potentially with better patient prognosis whereas lower levels were seen in the poorer prognostic NPI-3 group and higher grades. An opposite trend was seen in Aurora-C where lower levels were associated with the NPI-1 and NPI-2 groups and significantly higher levels were seen in the NPI-3 group. Aurora-B levels appeared to have no predictive function. This data suggests that together Aurora-A and Aurora-C levels, within a patient may be useful for predicting patient outcome.
P4-09-26
Prognostic Significance of Tissue Inhibitor of Metalloproteinase-1 (TIMP-1) in Breast Cancer.
Dechaphunkul A, Phukaloun M, Kanjanapradit K, Graham K, Ghosh S, Santos C, Mackey JR. Cross Cancer Institute, University of Alberta, Edmonton, AB, Canada; Faculty of Medicine, Prince of Songkla University, Hat Yai, Songkhla, Thailand

Background: Despite advances of systemic treatment in breast cancer guided by hormonal status and HER2 amplification, new prognostic and predictive factors are still warranted to optimize treatments among these patients. Tissue Inhibitor of Metalloproteinase-1 (TIMP-1), a physiologic inhibitor of Matrix Metalloproteinases (MMPs), can act in both pro- and anti-tumoral effects. The prognostic significance of TIMP-1 in breast cancer is still controversial. This study aims to determine the prognostic significance of TIMP-1 in breast cancer.

Material and Methods: One-hundred and seventy-six primary breast cancers from women with early stage disease treated with standard adjuvant therapy were analyzed by gene expression microarrays and immunohistochemistry for TIMP-1. Immunohistochemical analysis was independently reviewed by two pathologists.

Results: At the optimized cut-off point, patients with high TIMP-1 RNA levels had a significantly shorter time to relapse, with a hazard ratio (HR) of 1.6 (p = 0.039), but without significant differences in overall survival (HR 1.29, p = 0.37). Although cytoplasmic overexpression of TIMP-1 protein was not correlated with early relapse (HR 1.2, p = 0.35), high expression was associated with shorter overall survival (HR 1.73, p = 0.027). In multivariate analysis, when considering stage, histologic grade, hormonal and HER2 status, TIMP-1 RNA levels remained independently prognostic for early relapse (HR 1.68, p = 0.04).

Discussion: Elevated TIMP-1 RNA levels are independently prognostic for early recurrence, whereas protein overexpression of TIMP-1 is correlated with short overall survival in primary breast cancer.

P4-09-27
Can We Predict the Benefit of the 70-Gene Signature in the Choice of Adjuvant Systemic Treatment for ER Positive, HER2 Negative Tumors in Daily Practice? A Single Institution Experience.

Purpose: Studies have shown that the 70-gene signature (MammaPrint®) (MP) may outperform clinicopathological risk assessment and may predict the benefit from chemotherapy (CT) in patients (pts) with early-stage breast cancer. However, the need of fresh tissue and the high cost of the assay limit its use in daily clinical practice. We investigated whether 1) tumor clinicopathologic features can predict MP risk (high vs. low); 2) MP results could help to make decisions for the use of CT in pts with ER positive (ER+ve) breast cancer beyond recommendations of known international guidelines (NCCN, St. Gallen).

Patients and methods: Women with operable invasive breast cancer without evidence of distant disease undergoing surgery at the Breast Surgery Department were enrolled into the study. A 3 mm punch biopsy of the tumor was obtained from the specimen within the first hour after surgery. Samples were shipped to the laboratory in an RNA-stabilizing solution and were studied to ensure the presence of at least 30% of tumor cells and a customized microarray containing 70 genes was analyzed as described by the manufacturer.

Results: 124 consecutive pts were enrolled into the study; 106 tumor samples were adequate for the microarray. Median age was 53 yrs (range 28-83), mean tumor size was 2.3 cm (SD ±1.34), 52.4% pts had pN0, 55% of tumors had Ki-67 ≥20% and 36% were poorly differentiated. ER were detected in ≥50% of cells in 82% and <1% of cells in 15% of tumors, respectively; HER2 was positive in 18% of tumors.

As expected, poorly differentiated, ER and PgR negative, HER2 positive and highly proliferating tumors were more likely to be classified as high-MP. We then focused our analysis on ER and PgR+ve, HER2 negative tumors and assessed features correlated with MP results in this subgroup. Unexpectedly, 31/80 (39%) of these tumors were classified as high-MP vs. 49 (61%) low-MP. We found that tumor size (T1 vs. T2-T4), poor differentiation (G3 vs G1-2) and high proliferation (Ki-67 ≥20%) were significantly associated with a high-MP result. In an exploratory multivariate analysis tumor size and Ki-67 remained as independent predictors of high-MP result.

At last, when we compared MP risk with recommendations for AT from international guidelines we found that in the subgroup of candidates for endocrine therapy (ET) in whom the benefit from the addition of CT is undetermined, 25/68 pts (37%) were high-MP and 43 pts (63%) low-MP. When we considered recommendations for AT proposed by our multidisciplinary team according to international guidelines, 11/25 pts (44%) with high-MP received ET only and 14 pts (56%) CT + ET, while among 43 pts with low-MP only 9 received both CT and ET.

Conclusions: Our study shows that the 70-gene signature was feasible in the clinical setting, as 85% of tumor samples were adequate. A substantial proportion of ER/PgR+ve, HER2 negative tumors was classified as high-MP; within this subgroup, proliferation and tumor size independently predicted high-MP results. In 20 pts, MP risk would have resulted in discordant recommendations for AT compared to those based on standard clinicopathologic features.

P4-09-28
Comparison of Oncotype DX (ODX) and Mammostrat (MS) Risk Estimations and Correlations with Histologic Tumor Features in Low Grade, ER-Positive Invasive Breast Carcinoma (BC).
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Several molecular tests have been developed to estimate risk of distant recurrence (RDR) and help clinical decision-making regarding adjuvant chemotherapy in early stage BC. The ODX assay is a 21-gene expression profile mainly based on expression levels of genes related to hormone receptor / HER2 signaling and cell proliferation. MS is an immunohistochemistry-based assay measuring the expression of five markers thought to play a significant role in BC biology. Although both validated tests were shown to stratify patients into groups with low, intermediate and high RDR, the tests have not been compared head-to-head in the same cohort of patients and little data is available regarding their correlation with clinicopathologic tumor features.

We have previously shown that a proliferative, cellular stroma and inflammatory cells associated with tumor cells may account for unexpected intermediate/high risk estimations based on ODX in low grade BC. In this study we compared the clinicopathologic tumor features with risk estimations by ODX and MS in 106 low grade ER-positive BC. The histologic features of tumors were prospectively determined without knowledge of test results. The tumor stroma was evaluated for increased cellularity and presence of inflammatory cells. Double immunostain for pancytokeratin and
Ki67 was performed to assess cell proliferation in cancer vs stromal/inflammatory cells. Based on ODX and MS, among the 106 cases 68, 38 and 0, and 91, 14 and 1 tumors showed low, intermediate and high RDR, respectively. Assessment of the concurrence between the tests to predict low vs intermediate/high RDR showed a kappa value of 0.0541. There was no statistically significant correlation between ODX Recurrence Score (RS) and MS risk index values. We found no correlation between low vs intermediate/high risk estimation by either test and patient age, tumor size, nuclear atypia, mitotic rate, ER and HER2 expression levels. BC with intermediate/high RDR by ODX, but not by MS, showed significantly lower PR expression, increased stromal cellularity and presence of inflammatory cells. Double immunostains showed increased proliferation in stromal/inflammatory cells compared to cancer cells in cases showing intermediate/high RDR by ODX; no such association was seen with regards to MS risk estimations. The ratio of Ki67-positive stromal/inflammatory vs tumor cells >1 had an area under the curve of 0.8929 (p<0.0001) and 0.5026 (p=0.9823) to predict intermediate/high RDR based on ODX and MS, respectively. Cases showing intermediate/high RDR by ODX but low risk by MS were associated with increased stromal cellularity, presence of inflammatory cells and increased numbers of Ki-67 positive stromal/inflammatory cells, compared to cases showing low risk by both assays. Our results suggest that low grade ER-positive BC with increased stromal/inflammatory cell proliferation may show an apparent increased RDR as assessed by ODX, which uses RNA extracted from a mixture of tumor and stromal/inflammatory cells in the assay. MS, which examines cancer cells only (thus, not influenced by stromal and inflammatory cells), may provide a better estimation of likely tumor behavior in low grade BC.

P4-09-29
A Nomogram To Predict Prognosis in Node-Negative Breast Carcinoma.
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Background: Currently, the usefulness of conventional markers for breast carcinoma is limited to assess individual outcome. A statistical model was developed to improve the prognostic accuracy using multiple conventional and emerging prognostic biomarkers.

Methods: A total of 305 node-negative breast carcinomas who underwent surgery (+/- radiotherapy) but no adjuvant treatment was selected. Putative prognosis factors including age, tumor size, ER, PR, SBR grading, urokinase plasminogen activator (uPA), plasminogen activator inhibitor type 1 (PAI-1) and thymidine kinase (TK) were evaluated. The developed model was internally validated using Harrell’s concordance index and calibrated. An external validation of the new model is warranted.

Results: Age (p<0.001), PR (p=0.02), and PAI-1(p<0.001) were included in the Cox regression model predicting overall survival at 5-years. Internal validation revealed a concordance index of 0.711 to 0.694 before and after calibration.

Conclusion: A nomogram can be used to predict probability survival curves for individual breast cancer patients and the effect of treatment options can be evaluated using these models.

P4-09-30
Relationship between DNA Ploidy, Biomarker Expression and Molecular Subtypes of Invasive Breast Cancer.
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Background: DNA ploidy has been shown to have prognostic significance in patients with breast cancer. Studies in the past have mainly utilized flow cytometry (FCM) for measuring DNA ploidy. However FCM has several disadvantages, the instrument cannot distinguish benign from malignant cells and it cannot be performed on small tumor samples. The relationship between DNA ploidy and biomarker expression in breast cancer has not been well studied. Recently, gene expression analysis has demonstrated distinct subtypes of breast cancer. Expression of ER, PR and Her2 by IHC has been used as a surrogate tool for the molecular classification of breast cancer.

Aim: To determine the relationship between DNA ploidy, biomarkers (ER, PR, HER2, Ki67 and p53) expression and molecular subtypes of invasive breast cancer (IBC) using image analysis.

Design: DNA analysis was performed on Feulgen stained sections from the same tumor block used for biomarker analysis. DNA indices and ploidy were analyzed using the Autocyte Pathology Workstation (Tripath, Burlington, NC). Briefly, a total of 200-300 nuclei were collected and mean DNA index reported. DNA index was obtained by measuring the optical density of tumor cells in comparison with those of the non-neoplastic stromal cells in the sample using the latter as the diploid reference (value of 1.0). Tumors were classified as diploid (DNA indices of 0.90 to 1.1), aneuploid (DNA indices of <0.89 and > 1.1) and multiploid tumors; multiple indices. Patient’s age, tumor size, histologic grade, stage, biomarker status and breast cancer subtypes were correlated with ploidy status.

Results: Of the 248 cases of IBCA, 176 had aneuploid DNA and 72 diploid. Aneuploid tumors were predominantly grade 3, 72.1% versus 27.9% of diploid (p<0.0001). Tumor ploidy had no significant association with age (<40 years) versus older age (>50 years), p=0.118. The mean tumor size in aneuploid and diploid tumors was 4.2 cm and 3.2 cm respectively (p=0.009). Aneuploid tumors were frequently ER and PR negative compared to diploid (p<0.0001) with a mean ER level of 31.7% compared to 72.2% in diploid tumors (p<0.0001). The mean PR level was 17.2% in aneuploid versus 34.0% in diploid tumors (p=0.0009). The Ki67 index was 52.4% in aneuploid compared to 29.1% in diploid (p<0.0001). Tumor ploidy had no significant association with age (<40 years) versus older age (>50 years), p<0.0001. Tumor ploidy had no significant association with age (<40 years) versus older age (>50 years), p=0.118. The mean tumor size in aneuploid and diploid tumors was 4.2 cm and 3.2 cm respectively (p<0.0001). Aneuploid tumors were frequently ER and PR negative compared to diploid (p<0.0001) with a mean ER level of 31.7% compared to 72.2% in diploid tumors (p<0.0001). The mean PR level was 17.2% in aneuploid versus 34.0% in diploid tumors (p=0.0009). The Ki67 index was 52.4% in aneuploid compared to 29.1% in diploid (p<0.0001). The p53 expression was 30.3% in aneuploid versus 14.4% in diploid tumors. Aneuploid tumors were frequently of advanced T stage compared to diploid tumors (p=0.0048). There was no significant association with N stage (p=0.734). By multivariate analysis after adjusting for age, grade, T and N stage, ER (p=0.0021), PR (p=0.0003), HER2 overexpression (p=0.0028), Ki67 (p=0.0383), T stage (p=0.0172 and grade (<0.0001) were significantly associated with aneuploidy. Of breast cancer subtypes, Her2 (p=0.0001), triple negative (p<0.0001), luminal B subtype (p=0.006) were frequently aneuploid and luminal A was frequently diploid (p=0.051).

Conclusion: DNA ploidy measured by image analysis has significant association with biomarker expression. Increased DNA content is associated with poor prognostic features and aggressive subtypes of breast cancer.

P4-09-31
Withdrawn by Author

P4-09-32
Withdrawn by Author
**P4-09-33**  
The Prognostic Significance of Metaplastic Carcinoma of the Breast – A Comprehensive Comparison Study with Common Breast Cancers.  
Lai H-W, Kuo S-J, Chen D-R, Chen S-T. Changhua Christian Hospital, Changhua, Taiwan

**Purpose:** Metaplastic carcinoma of the breast (MCB) is a rare histological subtype of breast cancer with incidence less than 1%. Due to its rarity, the clinical characteristics and prognostic significance of MCB compared with other common breast cancer, such as infiltrating ductal carcinoma (IDC) and infiltrating lobular carcinoma (ILC) are not clear and controversial between different reports. We performed a multi-institutional cases collective comparison study to evaluate the clinical characteristics and prognostic status with IDC and ILC.

**Materials and methods:** Cases of MCB were enrolled from 4 medical centers in Taiwan. Forty-five MCB patients combined with 1777 IDC and 53 ILC patients from cancer registry database of Changhua Christian Hospital (CCH) comprised the current study. The patients’ demographic data, tumor characteristics and prognosis of MCB were analyzed and compared with IDC, and ILC. To further clarify the prognostic difference between MCB and IDC, a case control analysis was performed to minimize the effect of tumor size, lymph node status and other clinicopathological factors. The disease free survival (DFS) and overall survival (OS) between groups were compared.

**Results:** Compared with IDC, MCB was associated with older age, larger tumor size, lesser lymph node positive rate, higher distant metastasis, higher tumor grade, lower ER positive tumor, and higher triple negative breast cancer subtype (TNBC). Compared with IDC, MCB was associated with worse OS (p=0.049), but not different in DFS (p=0.132). To eliminate the influence of other prognostic factors, 135 cases controlled IDC with MCB. Both DFS and OS (p=0.132 and 0.289, respectively). To eliminate the influence of other prognostic factors, 135 cases controlled IDC with MCB. Both DFS and OS (p=0.132 and 0.289, respectively). To eliminate the influence of other prognostic factors, 135 cases controlled IDC with MCB. Both DFS and OS (p=0.132 and 0.289, respectively).

**Conclusion:** MCB was associated with poorer OS compared with IDC, and this poorer prognosis was related to tumor behavior rather than clinicopathologic factors.

**P4-09-34**  
Luminal Infiltrating Lobular Carcinoma of the Breast: Clinical and Prognostic Features.  

**Background:** Infiltrating lobular carcinoma (ILC) and infiltrating ductal carcinoma (IDC) are the two most common histologic types of invasive breast cancer. Previous studies have demonstrated distinctive clinical and biologic characteristics, however some features of ILC are yet uncertain like the relationship with the particular subtypes according to the gene expression profile. The purpose of our study is to analyze how the patients with ILC differ from IDC regarding of percentage of luminal subtype, the tumor factors, treatment and patterns of recurrence.

**Patients and Methods:** We assessed 1190 breast cancer patients treated at the Hospital Universitario 12 de Octubre between 1995 and 2006 categorized as having ILC or IDC pure types.

**Results:** One thousand one hundred ninety tumors were included, being ILC 223 patients (18%, 7%), IDC 967 patients (81%, 3%) and 200 patients (10%) were luminal HER2+. The current analysis is limited to the 990 patients classified as luminal HER2-.

**Conclusion:** Luminal HER2- Infiltrating lobular carcinoma carries distinct clinicopathological features. In our study, ILC had similar prognosis as IDC.
P4-09-35
Park S, Koo JS, Lee JS, Hwang H, Ko S, Kim SI, Park B-W, Yonsei University College of Medicine, Seoul, Korea

Background: The purpose was to investigate the implications of cyclooxygenase-2 (COX-2) and Ki-67 expression on the progression and prognosis of breast cancer.

Methods: COX-2 and Ki-67 levels were immunohistochemically determined using tissue microarrays of 1,028 breast tumors treated between November 1999 and August 2005. COX-2 and Ki-67 levels were compared by tumor progression and clinicopathological parameters were investigated in association with both markers status which was divided into 3 subgroups: COX-2(+)/Ki-67(+), COX-2(+)/Ki-67(-), COX-2(-)/Ki-67(any).

Results: COX-2 expression was significantly higher in intraductal carcinoma and progressively decreased in small T1 lesions and then in T2-T4 tumors, but Ki-67 was significantly expressed at high levels in T2-T4 lesions than in intraductal or T1 carcinomas. COX-2 expression was positively associated with favorable parameters such as older age at diagnosis, smaller size, well-differentiation, hormone receptor-positivity, and was inversely associated with Ki-67 expression. Among 816 invasive tumors, COX-2 expression alone was not related to outcomes. In combination with Ki-67 expression, however, COX-2(+)/Ki-67(-) tumors were significantly associated with favorable characteristics including HER2-negativity and demonstrated favorable outcomes. COX-2(+)/Ki-67(-) tumors showed unfavorable factors and the worst outcomes. COX-2(-)/Ki-67(+) subgroup presented intermediate prognosis. Statistical significance of COX-2 combined with Ki-67 was maintained in Cox’s models adjusting for clinicopathological parameters among not only whole population but also ER-positive subgroup.

Conclusions: Our study suggests that COX-2 is expressed in early phase of breast cancer development and is associated with favorable prognostic markers. But co-expression of high Ki-67 proliferative index could indicate an aggressive phenotypic change in COX-2 positive breast cancer. The mechanisms should be further investigated.

P4-10-01
Tumor Markers Predicting Recurrence Type after a Primary Ductal Carcinoma In Situ.
Zhou W, Johansson C, Jirström K, Ringberg A, Blomqvist C, Amini B-M, Fjällskog M-L, Wärnberg F. Uppsala University Hospital, Uppsala, Sweden; Malmö University Hospital, Malmö, Sweden; Lund University, Lund, Sweden; Helsinki University Central Hospital, Helsinki, Finland; Uppsala University, Uppsala, Sweden

Background: The risk for recurrence after a primary ductal carcinoma in situ (DCIS) is approximately 8% after ten years. If a recurrence occurs, the risk of the recurrence being invasive is approximately 50%. However, the knowledge of determinants for type of recurrence is limited. The purpose of this study was to investigate the predictable effect of specific biologic markers on the type recurrence after a primary DCIS.

Material and Methods: All women diagnosed with a primary DCIS between 1986 and 2004 in Uppsala and Västerås, Sweden were included (n=458). We also included all women from the SweDCIS Trial with a known recurrence (n=166). TMA blocks were constructed and immunohistochemistry (IHC) was used to evaluate the status of estrogen receptor (ER), HER2, epidermal growth factor receptor (EGFR/HER1), cytokeratin 5/6 (CK 5/6), forkhead box A1 (FOXA1), forkhead box C1 (FOXC1), Ki 67, CD 10 and transacting T-cell-specific transcription factor GATA-3 (GATA-3) in the primary DCIS tumors. Logistic regression was employed to calculate the difference of expression status of biological markers between groups. Odds ratios (ORs) and 95% confidence interval (CI) were calculated, with adjustment for age, free margins, surgical type and molecular subtype by IHC.

Results: Of the 624 patients, 358 without recurrence were grouped as controls. Of the 266 with recurrence, 130 developed an in situ recurrence, while the remaining 136 developed invasive recurrence. Mastectomy compared to breast conserving surgery (BCS) and free margins were associated with less recurrences (OR 0.07, 95% CI 0.03 – 0.15) and (OR 0.39, 95% CI 0.26 – 0.58). As compared with the most common molecular subtype Luminal A, the subtype Luminal B had statistically significant higher risk of recurrence (OR 2.01, 95% CI 1.12 – 3.59). Higher risk of all recurrences was also observed for FOXA1 (OR 3.16, 95% CI 1.48 – 4.74) and FOXC1 (OR 2.39, 95% CI 1.39 – 4.09). In the analyses of type of recurrence, ER-negative, HER2-positive and EGFR-positive tumors recurred more often as in situ (OR 2.34, 95% CI 1.17 – 4.71), (OR 2.08, 95% CI 1.11 – 3.88) and (OR 2.40, 95% CI 1.11 – 4.36). The molecular subtype HER2- was also associated with a lower risk of invasive recurrence (OR 0.27, 95% CI 0.11 – 0.67).

Conclusion: As expected, mastectomy and free margins were associated with a lower risk of recurrence. Molecular subtype Luminal B and tumor markers FOXA1 and FOXC1 had a higher risk of recurrence. Recurrences after molecular subtype HER2+, ER-negative and EGFR-positive DCIS were more often of the in situ type.

P4-10-02
A Meta-Analysis of the Association of Blood Levels of Vitamin-D and the Risk of Breast Cancer.
Amir E, Carlsson L, Seruga B, Ocana A, Goodwin PJ. Princess Margaret Hospital, Toronto, Canada; Institute of Oncology Ljubljana, Ljubljana, Slovenia; Albacete University Hospital, Albacete, Spain; Mount Sinai Hospital, Toronto, Canada

Background: A considerable body of literature has examined the association of vitamin-D with breast cancer risk and the potential role in its prevention. Geographic studies show higher incidence of breast cancer in patients residing at high latitudes. Other data linking vitamin-D deficiency to breast cancer risk are inconsistent.

Materials and Methods: A literature based meta-analysis was conducted. Odds ratios (OR) for breast cancer based on blood levels of 25-hydroxy or 1,25-hydroxy vitamin-D were computed and pooled. Analysis was conducted separately for studies where blood levels were taken before (group A) or after (group B) breast cancer diagnosis.

Results: Thirteen studies were identified. Nine studies were included in group A and 4 studies included in group B. For group A, there was no significant association between lower vitamin-D levels and breast cancer risk (pooled OR = 1.09, 95% confidence intervals 0.99-1.20, p=0.08). For group B, there was a highly significant association between lower vitamin-D levels and breast cancer (pooled OR = 2.81, 95% confidence intervals 1.70-4.65, p=0.001). The test for interaction between groups was highly significant (p<0.001). When all studies were pooled, the OR was 1.38 (95% confidence intervals 1.13-1.70, p=0.002).

Conclusion: When measured before breast cancer diagnosis, blood levels of vitamin-D are not associated with breast cancer risk. Breast tumors have been shown to differentially express vitamin-D hydroxylase. Therefore, any association of vitamin-D and breast cancer in studies measuring blood levels after breast cancer diagnosis may be confounded by reverse causation bias.
P4-10-03
Association between BMI, Physical Activity and Breast Cancer Histologic Types.
Nyante SJ, Dallal CM, Gierach GL, Sherman ME, Park Y, Hollenbeck AR, Brinton LA. National Cancer Institute, Rockville, MD; AARP, Washington, DC

Background: Previous research indicates that certain breast cancer risk factor associations vary by histologic tumor type. However, most studies have been too small to examine risk factors for uncommon histologic types. We examined the association between body mass index (BMI), physical activity, and the relative risk of breast cancer histologic types in a large prospective cohort study to determine whether associations for these risk factors varied by histology.

Methods: This analysis included women in the NIH-AARP Diet and Health Cohort Study who were 50 to 71 years old, postmenopausal and weight at baseline, and not previously diagnosed with cancer (N=190,348). The study cohort was established in 1995-1996 when participants completed a baseline questionnaire regarding health and nutritional information. BMI (kg/m²) was calculated from self-reported height and weight. Daily routine physical activity at work or home was derived from a question asking women what best described their daily routine at work, or throughout the day if they did not work at a job. Choices were mostly sitting with little walking, sitting with a fair amount of walking, mostly standing or walking, light lifting or climbing stairs/hills, or heavy work/carrying heavy loads. Cancer diagnosis and histology were obtained from state cancer registries. This analysis includes participant follow-up through December 31, 2006. After a median 11.2 years of follow-up, 7,631 invasive breast cancers were diagnosed: 5,278 ductal, 831 lobular, 640 mixed ductal-lobular, 214 mucinous, 132 tubular, and 536 other types. Multivariable-adjusted hazard ratios (HRs) and 95% confidence intervals (CIs) were estimated using Cox proportional hazards regression.

Results: Compared to women with a BMI of 18.5 to 24.9, obese women had increased risks of ductal (HR and 95% CI for BMI 30 to 34.9: 1.24, 1.14 - 1.36; BMI ≥ 35: 1.45, 1.30 - 1.62) and mucinous (HR and 95% CI for BMI 30 to 34.9: 1.58, 1.04 - 2.39; BMI ≥ 35: 1.93, 1.16 - 3.20) cancers. For both histologic types, the relative risk increased with increasing BMI (P-trend: ductal P<0.01; mucinous P<0.01). Women whose daily activity at work or home consisted of mostly standing or walking were at reduced risk of ductal and mixed ductal-lobular cancers compared with women who reported mostly sitting (HR and 95% CI for ductal: 0.86, 0.77 - 0.96; mixed-ductal lobular: 0.64, 0.48 - 0.86). Women who performed heavy work or carried heavy loads were also at a decreased risk of ductal and mixed ductal-lobular cancers compared with women reporting mostly sitting (HR and 95% CI for ductal: 0.68, 0.52-0.91; mixed ductal-lobular: 0.18, 0.04-0.72), though there were few mixed ductal-lobular cases who reported heavy activity.

Conclusions: High BMI was associated with increased risk of ductal and mucinous breast cancers. Women who spent their daily routine activity mostly standing or walking or doing heavy work had a reduced risk of ductal and mixed ductal-lobular cancers. These differences suggest that associations of BMI and physical activity vary by breast cancer histologic type in postmenopausal women and may have different roles in the etiology of these cancers.

P4-10-04
Automated Breast Cancer Risk Assessment: Identifying High Risk Women in the Primary Care Setting.
Ozanne E, Omer Z, Carlson K. University of California, San Francisco; Massachusetts General Hospital

Background: Despite the availability of risk assessment tools and risk-reducing interventions, high risk women are not routinely identified in clinical practice, and the few that are, rarely choose interventions – often due to misperception of risk. Identification of patients at risk needs to begin in the primary care setting, rather than the breast health specialty world. To accomplish this, we developed a web-based tool that provides automated risk assessment and personalized decision support designed for collaborative use between patients and clinicians in the primary care setting. We assessed the feasibility and efficacy of using this tool in a primary care clinic at an academic hospital to identify women at high risk.

Methods: Women aged 40 to 65 years of age were recruited from a schedule of patients attending annual physicals at a primary care clinic in an academic hospital. Patients with a history of breast cancer, genetic testing, or chemoprevention education were excluded. Information used to assess breast cancer risk was gathered from medical record review and phone interviews when necessary. Risk assessment was performed on all patients using four risk assessment models (Gail, Claus, BRCAPRO, and BCSC Density). Patients were randomized to view the decision aid either before their appointment or with their PCP during their appointment. Prior to each visit, providers received a risk report that summarized patient risk and recommended referrals. Outcomes were gathered from surveys administered to patients before and after appointments, and to providers after appointments.

Results: Over 4-months, 98 women were approached to reach the enrollment goal of 60 women (61%). 24/60 (40%) patients were identified to be high risk for breast cancer using standard high risk thresholds. 9/60 (15%) of patients fit criteria for referral to genetic counseling, while 15/60 (25%) fit the criteria for referral to a BC specialist. Out of the 24 patients who fit the referral criteria, 17 (71%) were referred to a high risk cliniculary by their PCPs. 9/17 (53%) patients followed through in scheduling the appointment within 4 months of the referral date. The PCPs’ perceptions of these patients’ risk was in line with the calculated risk for 21 (88%) of the patients. A discussion regarding breast cancer risk reduction occurred with 22/24 (92%) of these patients during the visit, while the PCPs chose to use the decision aid with only 13/24 (54%) of them. PCP chose to view the decision aid during the visit with more high risk patients than average risk patients (p=0.04). Use of the DA during the appointment did not alter provider satisfaction with visit. A majority of patients thought the DA was helpful in making a decision and would recommend it to women like them.

Conclusions: Performing personalized risk assessment and use of the decision aid in the primary care setting was feasible and acceptable. These results suggest risk assessment alone may be enough to encourage a discussion about breast cancer risk reduction for some providers. This method of risk assessment and decision support holds promise in the effort to reduce the incidence and burden of breast cancer.
**P4-10-05**

**Improved Breast Cancer Risk Assessment in Biopsied Women Using the Polyfactorial Model Oncovue®.**

*Jupé ER, Pugh TW, Knowlton NS, DeFreese DC. InterGenetics Incorporated, Oklahoma City, OK; NSK Statistical Solutions, LLC, Choctaw, OK*

**Background:** Regardless of the result, women that have undergone a breast biopsy are considered at increased risk due to the clinical circumstances leading to the need for the procedure. Both the number and outcome of biopsy are considered in the widely used Breast Cancer Risk Assessment Tool (BCRAT) also known as the Gail model. Accurately estimating individualized risk of developing breast cancer is useful for early detection and cancer prevention. Although the BCRAT has been demonstrated to accurately estimate the number of breast cancers likely to emerge in a population of women seeking regular mammography screening, it does not produce accurate individualized risk estimates for patient counseling. We have simultaneously analyzed gene polymorphism and clinical factor data in breast cancer cases and matched controls to develop a polyfactorial risk model (OncoVue) to improve estimation of individual risk. In three independent patient populations, OncoVue has been shown to significantly outperform both the BCRAT and composite risk scores produced by combining GWAS SNP risks with the BCRAT. Here we have characterized the performance of OncoVue in stratifying risk in women that have had one or more breast biopsies.

**Materials and Methods:** Risk scores were analyzed for participants ranging in age from 35 to 89 for a subset of participants that had enrolled in a larger case-control study conducted in six distinct geographic regions of the United States. The current study focused on the analysis of participants that had reported one or more biopsies (cases prior to diagnosis/controls at time of enrollment) amounting to 1265 Caucasian women (537 cases and 728 controls) in a model building set and 303 women in an independent validation set (134 cases and 169 controls). DNAs were genotyped for 22 SNP variants and genotype information was combined with clinical risk factor information to calculate the risk scores for the individual participants. Clinical factor information was also used to calculate BCRAT risk scores. The performance of OncoVue was examined in comparison to the BCRAT alone.

**Results:** For both models, positive likelihood ratios (PLR) were calculated as the proportion of patients with breast cancer with an elevated lifetime risk estimate (≥20%) divided by the proportion of disease-free individuals with an elevated risk estimate. In both the model building and validation sets, OncoVue exhibited approximately a 2.0-fold improvement compared to the BCRAT in more accurately assigning elevated risk estimates to breast cancer cases. In these women that are already considered at increased risk because of a history of biopsy, the observed level of improved performance of OncoVue was similar to that in our previous overall studies.

**Conclusions:** The OncoVue polyfactorial risk model incorporating both genetics and clinical factors improves on individualized breast cancer risk estimation compared to the BCRAT which uses only clinical factors. The performance in biopsied women further supports the potential utility of OncoVue for directing prevention and screening decisions.

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**P4-10-06**

**Evaluation of BRCAPRO Risk Assessment Model in Patients with Ductal Carcinoma In Situ.**

*Muse KI, Elsayegh N, Gutierrez-Barrera AM, Kerber H, Valero V, Litton JK, Hortobagyi GN, Arun BK. UT MD Anderson Cancer Center, Houston, TX*

**Background:** The BRCAPRO model is used to predict a patient’s likelihood to possess a BRCA1 or BRCA2 gene mutation based upon personal and family history. Ductal carcinoma in situ (DCIS) is considered a non-invasive condition which can progress to an invasive breast cancer if left untreated. Currently, DCIS is not specifically accounted for in the BRCAPRO model, thereby causing DCIS to be weighted in the same manner as an invasive breast cancer diagnosis. Historically, a diagnosis of DCIS has been entered as having developed into an invasive breast cancer ten years later. However, there are no standard guidelines of how DCIS should be entered. We sought to determine if there were any differences in how DCIS was treated in the BRCAPRO model to predict the more effective method in calculating the BRCAPRO. Methods: Women with pure DCIS, who were referred for genetic counseling and underwent genetic testing, were included in the study. The likelihood of carrying a BRCA mutation was calculated using the BRCAPRO model (Version 5.1). Patient characteristics which were entered into the BRCAPRO model include: tumor markers (estrogen receptor-ER and progesterone receptor-PR), history of oophorectomy prior to diagnosis, family history of 1st and 2nd degree relatives with breast and ovarian cancer, race and Ashkenazi Jewish ancestry. Each patient’s BRCAPRO risk estimate was calculated and compared by entering DCIS at the presenting age of diagnosis and by adding 10 years to the age of diagnosis. Descriptive statistics and a student’s t-test were used to compare BRCAPRO estimates between the two groups. Results: Ninety-five patients with pure DCIS underwent genetic counseling and testing. The average age of DCIS diagnosis was 45 years (range 26-65). Of the 95 DCIS patients included in the study 21% (n=20) tested positive for a BRCA gene mutation (8 BRCA1 and 12 BRCA2), 77% (n=74) test negative and 0.01% (n=1) had a variant of uncertain significance. Overall, DCIS patients who tested positive for a BRCA mutation had a higher BRCAPRO (40%) than patients who tested negative (12%) when presenting age of diagnosis was assessed. When 10 years was added, the BRCAPRO estimate was still higher amongst BRCA positive patients (28%) than BRCA negative patients (8%). The mean BRCAPRO probability when DCIS was entered at presenting age of diagnosis was 18% (0.1-95.4) versus 12% (0.1-89.9) when calculated 10 years later. Conclusion: In our cohort there was no significant difference in BRCAPRO probability whether DCIS was entered at the presenting age of diagnosis or 10 years later (p=0.1). However, future studies are needed to determine the most effective method to incorporate DCIS into the BRCAPRO model in order to determine those individuals who may or may not be at increase risk to possess a BRCA gene mutation.
P4-10-07
Clinico-Pathological Features of Breast Cancer Patients with Primary Metastatic Disease Versus Localized Disease: A Multicenter Study.
Barinoff J, Hils R, Bender A, Gross J, Kurz C, Tauchert S, Mann E, Schwidde I, Ipsen B, Sawitzki K, Heitz F, Harter P, Traut A, du Bois A. Kliniken Essen Mitte, Essen, Germany; Dr.-Horst-Schmidt-Klinik, Wiesbaden, Germany; Asklepions Klinik, Lich, Germany; Sankt Gertrauden-Krankenhaus, Berlin-Wilmersdorf, Germany; Klinikum Esslingen, Esslingen am Neckar, Germany; CaritasKlinik St. Theresia, Saarbrücken, Germany; Universitätssklinikum Gießen und Marburg, Standort Marburg, Marburg, Germany; Klinikum Frankfurt Hôchst, Frankfurt am Main, Germany; Evangelisches Krankenhaus Wesel, Wesel, Germany

Objective: Approximately 6% of breast cancer patients present with primary metastatic disease (pmBC) at first diagnosis. Clinico-pathological differences to non-metastatic patients are undetermined.

Methods: Exploratory analysis of patients with pmBC treated in 8 breast cancer units between 1998 and 2010. Tumor characteristics of these patients were compared with non-metastatic breast cancer patients (BC) of one breast cancer center who had neither local-recurrence nor distant metastases during 30 months of follow-up after first diagnosis. Standard staging in patients with first recurrence nor distant metastases during 30 months of follow-up. These patients were compared with non-metastatic breast cancer patients of one breast cancer center who had neither local-recurrence nor distant metastases during 30 months of follow-up.

Results: 2,214 patients were included, 1,642 with BC and 572 with pmBC, respectively. Patients with pmBC were 7 years older (BC 58 years of age vs. pmBC 65 years; p=0.000) and were more likely to be postmenopausal (74% vs. 83%; p=0.000). Most common localizations of distant first metastases were bone (61.5%), liver (24%), lung (21%) and non-axillary lymph nodes (12%).

85 (15%) patients with pmBC were diagnosed in stage T1. Factors associated with pmBC in multivariate analysis for this group were positive lymph node status (OR 4.8; 95%CI 1.1-13.0; p=0.017) and grading 3 (OR 1.6; 95% CI 1.1-2.3; p=0.019) were reported as risk factors for this group. 90 (16%) and 200 (35%) patients were diagnosed with stages T3 and T4, respectively. In T3/4 tumors a positive lymph node status (OR 5.2; 95% CI 2.9-9.3; p=0.000) and grading 3 (OR 2.2; 95% CI 1.2-3.9; p=0.009) could be defined as significant risk factors for distant metastases.

Postmenopausal status was associated with primary metastases in stage T2; positive lymph node status (OR 4.8; 95%CI 1.1-13.0; p=0.017) and grading 3 (OR 1.6; 95% CI 1.1-2.3; p=0.019) were reported as risk factors for this group. 90 (16%) and 200 (35%) patients were diagnosed with stages T3 and T4, respectively. In T3/4 tumors a positive lymph node status (OR 5.2; 95% CI 2.9-9.3; p=0.000) and grading 3 (OR 2.2; 95% CI 1.2-3.9; p=0.009) could be defined as significant risk factors for distant metastases.

Conclusion: The clinico-pathological features of breast cancer patients with or without primary metastases differ. In all stages positive lymph node status and higher grading were associated with pmBC significantly. Lobular histology was reported as a risk factor for T1-2 compared to patients without metastases. This feature was not found for T3/4 pmBC. T1 pmBC were likely to be associated with luminal B phenotype. T3-4 pmBC have not been associated with any phenotype or hormone receptor constellation as risk factor for metastases. Tumor biology seems to play a minor role for risk of metastases in T3-4 stages compared to patients with T1-tumors. Findings from this analysis should be considered in the choice of staging methods, especially in stage T1.

P4-10-08
Risk Factor Profiles of Women with DCIS and Invasive Ductal Breast Cancers.
Schnabel F, Chun Kim J, Billig J, Cimeno A, Mehta P, Gudh A, Hochman T, Shapiro R, Hoots K, Siegel B, Axelrod D. NYU Cancer Institute, NYU Langone Medical Center, New York, NY; NYU Langone Medical Center, New York, NY

Background
There are well established risk factors for breast cancer, including elements of personal and family history. Ductal carcinoma in situ (DCIS) and invasive ductal carcinoma (IDC) have long been viewed as representing two points on the spectrum of one disease. There is limited and conflicting information in the literature regarding risk factor profiles for DCIS as compared with IDC. The purpose of this study was to investigate the relationship of established risk factors in a population of women newly diagnosed with pure DCIS and IDC.

Methods
The Breast Cancer Database at NYU Langone Medical Center was queried for women who were diagnosed with pure DCIS and IDC from 1/2010-3/2011. Variables of interest included: age, family history of breast cancer (FHBC), BRCA1/2 status, age at menarche and menopause, parity, age at first birth, breast feeding, body mass index (BMI), history of atypical hyperplasia or lobular carcinoma in situ (ADH, ALH, LCIS), stage, ER/PR status, and method of presentation. Wilcoxon non-parametric tests, Chi-Square tests, and Fisher’s Exact Tests were used to evaluate differences among DCIS and IDC patients.

Results
Of the 593 women identified in this study, 140 (24%) had pure DCIS and 453 (76%) had IDC. The median age at diagnosis was 59 years. There were 9 (1.5%) BRCA1/2 mutation carriers. Of these, 7 had IDC and 2 had DCIS. The majority of patients with IDC were stage I (67%) and ER/PR+ (73%). The majority of patients with DCIS were also ER/PR+ (71%). In our cohort, the majority of DCIS (83%) was mammographically detected versus 47% of IDC cases. There were no statistically significant differences in breast cancer risk factors between the two groups.

Table 1: Clinical Characteristics of Study Cohort with DCIS and IDC

<table>
<thead>
<tr>
<th>VARIABLES</th>
<th>DCIS (N=140)</th>
<th>IDC (N=453)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>age (median+range)</td>
<td>59 (40-95)</td>
<td>59 (40-95)</td>
<td>0.15*</td>
</tr>
<tr>
<td>FHBC</td>
<td>&lt;0.001^</td>
<td>67 (48%)</td>
<td>217 (48%)</td>
</tr>
<tr>
<td>TORSO</td>
<td>3 (1.7%)</td>
<td>236 (52%)</td>
<td>0.003*</td>
</tr>
<tr>
<td>ADH/ALH</td>
<td>6 (4.3%)</td>
<td>118 (26%)</td>
<td>0.03*</td>
</tr>
<tr>
<td>LUMINAL B (median+range)</td>
<td>31 (9-58)</td>
<td>51 (29-63)</td>
<td>0.24*</td>
</tr>
<tr>
<td>PARITY</td>
<td>2 (1.5%)</td>
<td>134 (30%)</td>
<td>0.05*</td>
</tr>
<tr>
<td>0</td>
<td>2 (1.5%)</td>
<td>61 (13%)</td>
<td>0.73</td>
</tr>
<tr>
<td>1</td>
<td>2 (1.5%)</td>
<td>61 (13%)</td>
<td>0.73</td>
</tr>
<tr>
<td>&lt;2</td>
<td>1 (0.7%)</td>
<td>58 (13%)</td>
<td>0.73</td>
</tr>
<tr>
<td>&gt;2</td>
<td>1 (0.7%)</td>
<td>58 (13%)</td>
<td>0.73</td>
</tr>
<tr>
<td>AGE AT 1ST BIRTH</td>
<td>26 (16-34)</td>
<td>26 (16-34)</td>
<td>0.26*</td>
</tr>
<tr>
<td>WEIGHT</td>
<td>3 (5%)</td>
<td>168 (39%)</td>
<td>0.003*</td>
</tr>
<tr>
<td>BMI (median+range)</td>
<td>45 (34-63)</td>
<td>44 (34-63)</td>
<td>0.06*</td>
</tr>
<tr>
<td>0</td>
<td>54 (5%)</td>
<td>117 (26%)</td>
<td>0.003*</td>
</tr>
<tr>
<td>&lt;2</td>
<td>244 (17-342)</td>
<td>255 (16-355)</td>
<td>0.20*</td>
</tr>
<tr>
<td>≥2</td>
<td>13 (1%)</td>
<td>41 (9%)</td>
<td>0.60*</td>
</tr>
<tr>
<td>no</td>
<td>69 (49%)</td>
<td>258 (57%)</td>
<td>0.14</td>
</tr>
<tr>
<td>≥2</td>
<td>69 (49%)</td>
<td>258 (57%)</td>
<td>0.14</td>
</tr>
<tr>
<td>ever</td>
<td>45 (49%)</td>
<td>255 (57%)</td>
<td>0.26*</td>
</tr>
<tr>
<td>menopausal status</td>
<td>135 (96%)</td>
<td>446 (98%)</td>
<td>0.017***</td>
</tr>
<tr>
<td>abs</td>
<td>7 (5%)</td>
<td>18 (4%)</td>
<td>0.15*</td>
</tr>
<tr>
<td>METHOD OF PRESENTATION</td>
<td>5 (4%)</td>
<td>7 (2%)</td>
<td>0.80</td>
</tr>
<tr>
<td>palpable finding</td>
<td>7 (8%)</td>
<td>30 (6%)</td>
<td>0.0004*</td>
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<tr>
<td>mammography</td>
<td>130 (85%)</td>
<td>365 (74%)</td>
<td>0.27</td>
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<tr>
<td>ultrasound</td>
<td>5 (4%)</td>
<td>32 (6%)</td>
<td>0.003*</td>
</tr>
<tr>
<td>MRI</td>
<td>5 (4%)</td>
<td>32 (6%)</td>
<td>0.003*</td>
</tr>
<tr>
<td>Other 2 (1%)</td>
<td>9 (2%)</td>
<td>16 (3%)</td>
<td>0.003*</td>
</tr>
</tbody>
</table>

*Wilcoxon Non-Parametric Test, ^Pearson’s Chi-Square, ^^Fisher’s Exact Test

Conclusions
In contrast to some previously published work, the risk factor profile in our cohort was similar for patients with IDC and DCIS. In this population, the majority of cases of DCIS were detected mammographically, underscoring the importance of mammography in early detection of breast cancer. There is increasing interest in identifying a population of women with DCIS who might never progress to invasive disease. Our data suggests that this population...
is unlikely to be distinguished by its risk factor profile. Genetic and molecular characteristics are more likely to provide the criteria by which this subgroup of patients is identified.

P4-10-09
Hormone Replacement Therapy: A Benign Breast Disease’s Risk?

Background
Use of hormone replacement therapy (HRT) has been associated with increased risk of breast cancer. However, relations between use of HRT and benign breast lesions remain contradictory (1-3). Our goal was to investigate whether HRT and its duration use are associated with benign breast lesions.

Materials & Methods
From 2001 to 2007, 2708 patients with a non palpable breast lesion were referred to our breast care center. Radiological abnormalities were screened either by breast ultrasound mammography, or MRI. Of those, 1,329 patients had a biopsy in our Radiology Unit. We focus on the postmenopausal women (n=739). Clinical data including HRT use extent were reported. Patients with previous history of benign breast disease or malignancy were excluded.

Results
The analysis was performed on 739 patients. Biopsies yielded a benign result in 414 (56%) of them (fibroadenoma n=71, blunt duct adenosis n=94, fibrocystic disease n=92, epithelial hyperplasia n=56, others n=101), high risk lesions in 57 (8%) (atypical ductal hyperplasia n=28, atypical lobular hyperplasia n=19, lobular carcinoma in situ n=10) and malignancy in 268 (36%) (not studied here). For the 471 patients with benign or high risk breast lesion, the median age was 59 years, 80% had at least one pregnancy and 27% had a family history of breast cancer. Among these 471 patients, 47% of them had used an oral contraceptive and 53% of them a HRT, with a mean duration of use of 2 years.

The HRT use was not significantly associated with the type of benign or high risk breast disease. No association was observed either between breast disease and duration of HRT (less or more than 7 years). Older age was significantly correlated to atypia or carcinoma (median age at diagnosis: 59 y.o in benign lesions, 60 y.o in high risk lesions and 62 y.o in malign lesions, with significant difference) (p<0.0001).

Conclusion
In this study of postmenopausal women, HRT and its duration use were not associated with the type of benign or high risk breast lesion. Although a lack of statistical power may be invoked to explain the results, we are called to believe that the magnitude of an effect of HRT use is small, if ever it exists.


P4-10-10
Breast Cancer Risk Factors among Asian Versus Caucasian Women with BRCA1/2 Mutations.
de Bruin MA, Kwong A, Goldstein BA, Lipson JA, Ikeda DM, McPherson LA, Sharma B, Kardashian A, Schackmann EA, Kingham KE, Mills MA, West DW, Ford JM, Kurian AW. Stanford University School of Medicine, Stanford, CA; University of Hong Kong Li Ka Shing Faculty of Medicine, Hong Kong, China

Background: Prior research suggests that the prevalence and penetrance of BRCA1/2 mutations may be different in Asians compared with Caucasians. Such differences in penetrance could be related to genetic, as well as hormonal, reproductive and lifestyle factors.

Methods: Chinese and Caucasian BRCA1/2 mutation carriers were recruited from genetics clinics in Hong Kong and California. We compared personal and family history of cancer, as well as hormonal, reproductive and lifestyle exposures between racial groups. Breast density was estimated using BI-RADS breast composition categories on mammogram. We analyzed DNA samples for single-nucleotide polymorphisms (SNPs) that may modify BRCA-associated cancer risk, and compared the prevalence between groups. A multivariable analysis of potential modifiers of cancer risk is underway.

Results: Forty-two Chinese women from Hong Kong and forty-nine Caucasian women from California were enrolled. BRCA1 mutations were more common in Caucasian women (63% vs. 43%, P=0.05), while BRCA2 mutations were more common among Chinese women (57% vs. 33%, P=0.02). More Chinese women had a personal history of breast cancer (91% vs. 53%) and ovarian cancer (12% vs. 4%). In contrast, significantly more Caucasian women had a family history of breast and ovarian cancer. On average, Caucasian women had higher parity, breastfed more, had less dense breasts on mammogram, and more often underwent prophylactic oophorectomy, all of which are protective against breast cancer in the general population. However, Caucasian women also consumed more alcohol and had higher average BMI. Risk associated alleles in RAD51, MAP3K1 and TOX3/TNRC9 were more common in Chinese women; risk associated alleles in FGFR2 and RASSF1 were similar in frequency between groups. Results of the multivariable analysis are pending and will be presented.

Discussion: We found notable differences between Chinese and Caucasian women in the proportion of BRCA1 versus BRCA2 mutations, and in the distribution of hormonal, reproductive and lifestyle factors, and SNPs associated with breast cancer risk. Such variations in risk-modifying factors may contribute to differences in BRCA mutation penetrance. Enhanced understanding of racial differences in BRCA1/2 mutation epidemiology may inform targeted cancer screening and prevention strategies.
demonstrated that this marked increase is mostly due to an increase in the estrogen receptor (ER)-positive subtype. It is necessary to establish risk factors capable of predicting the risk of ER-positive breast cancer which will enable the efficient selection of candidates for preventive chemotherapy. We analyzed genetic factors, including 14 single nucleotide polymorphisms (SNPs), environmental risk factors (body-mass index (BMI), age at menarche, pregnancy, age at first birth, breastfeeding, family history of breast cancer, age at menopause, use of hormone replacement therapy, alcohol intake and smoking), serum hormones and growth factors (estradiol, testosterone, prolactin, insulin-like growth factor 1 (IGF1) and IGF binding protein 3 (IGFBP3)) and mammographic density in 913 women with breast cancer and 278 disease-free controls. To identify important risk factors, risk prediction models for ER-positive breast cancer in both pre- and postmenopausal women were created by logistic regression analysis. In premenopausal women, 1 SNP (CYP19A1-rs10046), age, pregnancy, breastfeeding, alcohol intake, serum levels of prolactin, testosterone and IGFBP3 were considered to be risk predictors. In postmenopausal women, 1 SNP (TP53-rs1042522), age, BMI, age at menopause, serum levels of testosterone and IGF1 were identified as risk predictors. Risk factors may differ between women of different menopausal status, and inclusion of common genetic variants and serum hormone measurements as well as environmental factors might improve risk assessment models. Further validation studies will clarify appropriate risk groups for preventive chemotherapy.

**P4-11-01**

Bilateral Oophorectomy Is Associated with a Higher Prevalence of Arthritis and Lower Bone Mineral Density in Women 40 Years and Older.

McCarthy AM, Visvanathan K. Johns Hopkins Bloomberg School of Public Health, Baltimore, MD; Johns Hopkins Sidney Kimmel Comprehensive Cancer Center, Baltimore, MD

**Background:** BRCA 1/2 mutation carriers are encouraged to have their ovaries removed by age 40 to reduce cancer risk and prolong survival. Given the recency of genetic testing, it will be some years before robust estimates are available of the long term adverse effects. In the meantime data from the general population can help inform women and providers about potential adverse effects so that preventive strategies can be implemented. Bone loss accelerates following natural menopause and oophorectomy before age 45 is a known risk factor for osteoporosis. However there is limited data quantifying the effects of bilateral oophorectomy on bone mineral density (BMD), an early sensitive marker of osteoporosis.

**Methods:** We evaluated the associations of oophorectomy with arthritis and BMD in NHANES III, a nationally representative survey conducted 1988-1994. For analysis we included women aged 40 years and older with no history of cancer who reported a bilateral oophorectomy or intact ovaries. Women were asked if a doctor told them they had arthritis. Femoral neck BMD was measured by dual energy x-ray (DXA). Osteoporosis was defined as BMD (g/cm^2) more than 2.5 standard deviations below the mean of white women aged 20-29 years. Survey weights were used to account for the complex survey design. Odds Ratios (OR) for arthritis and osteoporosis were estimated using logistic regression. Oophorectomy status was categorized into intact ovaries, oophorectomy <45 years, and oophorectomy ≥45 years. Multivariate models were adjusted for age at interview, race, income, smoking status, education, alcohol, and BMI. Analyses were repeated excluding women who reported using hormone replacement therapy (HRT).

**Results:** The sample size was 4039 for the arthritis analysis and 3660 for the BMD analysis. Women with oophorectomy were more likely to report arthritis than women with intact ovaries (45.4% vs. 32.1%; p<0.001). The prevalence was even higher in women with oophorectomy <45 years (47.7%). The age-standardized mean femoral neck BMD was lower for women reporting oophorectomy at <45 years than women with intact ovaries (0.711 vs. 0.743 g/cm^2; p=0.017). In multivariate models women with oophorectomy had greater odds of arthritis than women with intact ovaries, particularly among women with oophorectomy <45 years (OR=1.78 95% CI 1.31-2.42). The odds of arthritis were even greater after excluding HRT users (OR=1.99 95% CI 1.25-3.18). Similarly, women who had a prior oophorectomy had greater odds of osteoporosis than women with intact ovaries, particularly among women with oophorectomy <45 years (OR=1.78 95% CI 1.07-2.97). The odds were increased further after excluding HRT users (OR=2.92 95% CI 1.32-6.44).

**Conclusions:** The prevalence of arthritis and osteoporosis were significantly greater in women who reported bilateral oophorectomy. Results were most profound among women whose oophorectomy was performed before age 45 and among women who never used HRT. These results suggest that women who undergo oophorectomy for cancer prevention should be closely monitored for osteoporosis over the long term.

**P4-11-02**

Insulin-Like Growth Factor-1 (IGF-1), Insulin-Like Growth Factor Binding Protein-3 (IGFBP-3) and Lobule Type among Women in the Nurses' Health Study II (NHS II).

Collins LC, Rice MS, Shen D, Connolly JL, Schnitt SJ, Tamimi RM. Beth Israel Deaconess Medical Center and Harvard Medical School; Brigham and Women’s Hospital and Harvard School of Public Health, Boston

In a previous analysis of women enrolled in NHSII, we found that among women diagnosed with benign breast disease (BBD), those with predominant type 1/no type 3 lobules were at lower risk of subsequent breast cancer compared to women with other lobule types. Additionally, studies in animal models suggest that higher levels of IGF-1, a polypeptide hormone involved in the proliferation/differentiation of normal mammary epithelium, may inhibit involution of breast lobules. However, the interaction between IGF-1 levels and lobule types in determining breast cancer risk has not been previously evaluated. Therefore, we examined the association between IGF-1 levels and lobule type among women with BBD.

**Methods:** We conducted a cross-sectional study among 484 women in NHSII with biopsy-confirmed BBD between 1993-2001 who had blood samples available for determining levels of IGF-1 and IGFBP-3. A pathologist, blinded to exposure status, classified lobule type on biopsy slides according to the number of acini per lobule (type 1 = 12; type 2=50; type 3~80 acini). Lobule type was classified into (1) predominant type 1/no type 3 lobules or (2) other lobule types. Multivariate logistic models were used to assess the associations between plasma IGF-1, IGFBP-3, and IGF-1/IGFBP-3 levels with lobule type. Models were adjusted for age, IGF-1 batch and additional potential confounders in secondary analyses.

**Results:** In univariate analyses, older age at biopsy, higher body mass index, postmenopausal status, nulliparity, and lower IGF-1 levels were associated with predominant type 1/no type 3 lobules (p<0.05). In multivariate logistic models adjusting for age, higher IGF-1 levels were associated with a decreased risk of predominant type 1/no type 3 lobules (OR quartile 4 vs. quartile
1 = 0.35, 95% CI: 0.15-0.81). Greater IGF-1/IGFBP-3 ratio was also associated with a decreased risk of predominant type 1/no type 3 lobules (OR quartile 4 vs. quartile 1 = 0.24, 95% CI: 0.10-0.57).

IGF-1/IGFBP-3 Ratio and Lobule Type.

Model 1: Age and batch

Quartile 1 (30/91) Quartile 2 (20/101) Quartile 3 (19/102) Quartile 4 (9/112)

p-value

0.60 (0.30, 1.21) 0.59 (0.28, 1.19) 0.24 (0.10, 0.57) <0.01

Model 2: Model 1 + BMI, Menopausal Status, Histological categories

Quartile 1 (30/91) Quartile 2 (30/96) Quartile 3 (30/93) Quartile 4 (9/111)

p-value

0.74 (0.36, 1.55) 0.73 (0.33, 1.60) 0.34 (0.13, 0.86) 0.07

Model 3: Model 2 + parity, alcohol

Quartile 1 (30/91) Quartile 2 (30/96) Quartile 3 (30/93) Quartile 4 (9/111)

p-value

0.79 (0.38, 1.66) 0.79 (0.36, 1.75) 0.34 (0.13, 0.86) 0.06

Outcome is predominant type 1/no type 3 lobules (complete involution)

These associations persisted, though were slightly attenuated, in models adjusting for additional potential confounders. **Conclusion:** Higher IGF-1 levels and greater IGF-1/IGFBP-3 ratios are associated with a decreased risk of predominant type 1 lobules/no type 3 lobules among women with BBD in the NSHII. Whether this association contributes to the mechanism by which IGF-1 confers an elevated breast cancer risk requires further investigation.

**Acknowledgements:** This work was supported by T32 CA09001-35 CA124865, R01 CA050385, and the Breast Cancer Research Foundation

**P4-11-03**

**Prognosis of Pregnancy-Associated Breast Cancer: A Meta-Analysis Involving 39,415 Patients.**

Azim, Jr HA, Santoro L, Russell-Edu W, Pentheroudakis G, Pavlidis N, Peccatori FA. Institut Jules Bordet, Brussels, Belgium; European Institute of Oncology, Milan, Italy; University of Ioannina, Ioannina, Greece

**Background:** Pregnancy-associated breast cancer (PABC), defined as breast cancer diagnosed during pregnancy or one year after, is a relatively rare disease and hence considerable controversy exists regarding its prognosis compared to non-pregnancy-related breast cancer.

**Methods:** Two of the authors independently performed a literature search on MEDLINE and Cochrane Library with no date or language restrictions. Eligible studies were control-matched, population-based and hospital-based studies that have addressed the outcome of patients diagnosed during pregnancy or one-year afterwards. The control group was defined as patients diagnosed with breast cancer not related to pregnancy. The primary and secondary end-points were overall and disease-free survival respectively. Pooling of data was done using the random effect model. To control for potential differences between the two groups in systemic treatment and clinico-pathological features (e.g. tumor size, nodal status, ER, etc...) that could affect prognosis, we performed a sensitivity analysis by pooling the hazard ratios (HRs) of the adjusted multivariate models within each study. We also analyzed differences according to time of diagnosis (during pregnancy or post-partum), type of study and year of reporting the study. Finally, we communicated with the authors of the eligible studies to collect unpublished statistics relevant to our analysis to further refine our findings.

**Results:** 29 studies were included in this meta-analysis (2903 cases and 36,512 controls). Women diagnosed with PABC had a significantly higher risk of death compared to those diagnosed with non-pregnancy-related breast cancer (pooled hazard ratio (pHR): 1.47 [95% CI: 1.30-1.65]). A sensitivity analysis including 11 studies (1222 PABC cases, 19231 controls) adjusted for differences in tumor size, nodal status and systemic treatment showed the same result (pHR: 1.44 [95% CI: 1.17-1.77]). These findings were consistent in patients diagnosed either during pregnancy (pHR: 1.30 [95% CI: 1.01-1.67]) or in the post-partum period (pHR: 1.56 [95% CI: 1.08-2.26]) with no heterogeneity observed (p=0.43). Sensitivity analyses according to the type and year of study showed the same findings. Regarding the secondary end-point, only ten studies (531 cases, 1842 controls) provided sufficient information to estimate disease-free survival, and indeed PABC patients had a higher risk of relapse compared to breast cancer controls (pHR: 1.59 [95% CI: 1.23-2.07]). Collection of unpublished data is currently ongoing and further analyses will be presented at the meeting.

**Conclusion:** To the best of our knowledge, this is the largest analysis addressing the prognosis of PABC. Our results confirm that PABC is independently associated with a worse prognosis whether diagnosis is made during pregnancy or in the post-partum period. This underscores the possible impact of pregnancy on breast cancer biology. In this regard, we are currently interrogating potential biological differences between PABC patients and matched breast cancer controls at the gene expression level to elucidate the biology of this relatively rare, yet very challenging disease.

**P4-11-04**

**Risk of Primary (PBC) and Contralateral Breast Cancer (CBC) after Ovarian Cancer (OC) in BRCA1 and BRCA2 Mutation Carriers: Implications for Surveillance and Risk Reducing Mastectomy.**


**Background:** In view of the increased risk of developing breast (BC) cancer, BRCA mutation carriers are offered intensive surveillance or risk reducing mastectomy (RRM) and/or salpingo-oophorectomy (RRSO). It is insufficiently clear how to counsel mutation carriers who have been treated for OC, and it may be questioned whether RRM is indicated. It is possible that BC risk, both of primary (PBC) and contralateral BC (CBC), may be modified by the treatment given for OC mostly including surgery and platinum-based chemotherapy. Further, the BC risk has to be weighted against the risk of death after OC. So far, there are no data available on the PBC or CBC risk after OC in BRCA mutation carriers.

** Patients and Methods**

From the database of the institutional Family Cancer Clinic (FCC), we selected BRCA1-associated OC patients either without (n= 79, at risk of PBC) or with a history of unilateral BC (n=37, at risk of CBC). Controls were BRCA mutation carriers without OC, either without (n=351) or with a history of unilateral BC (n=294). Follow-up started at OC diagnosis, and for controls at date of first visit at the FCC or the 35th birthday. Exclusion criteria were: other malignancy besides epithelial OC or BC, inadequate follow-up data, bilateral breast cancer or mastectomy. Data analyses were performed using t- and chi-squared tests, and Kaplan-Meier survival method with death prior to BC as competing risk event.

**Results**

Only six OC patients (5.1%) did not receive chemotherapy as part of primary therapy for OC, five at risk of PBC and one at risk of CBC. Chemotherapy schedules were mainly platinum-based (92%). RRSO was performed in 45% of the controls. The risks of PBC at 2, 5, and 10 years in BRCA-associated OC patients were 3%, 6% and 11%, respectively, versus 6%, 16% and 28% in unaffected BRCA mutation carriers (p=0.03). The mortality...
rate at those time points in the OC group was 13%, 33% and 61%, respectively, versus 1%, 2% and 2% in unaffected mutation carriers (p<0.001). In patients with a history of unilateral BC, the risks of CBC at 2, 5, and 10 years in OC patients were 0%, 7% and 7%, respectively, versus 6%, 16% and 34% in the BC patients without OC (p=0.06). The mortality rate at similar time points was 19%, 34%, and 55%, respectively, in the OC group versus 4%, 11%, and 21% for the non-OC patients (p<0.001).

Conclusions
The risk of developing a PBC or CBC was much lower in BRCA mutation carriers with a history of OC than in mutation carriers without OC. Also, since the 10-year mortality rate after ovarian cancer was 55-60% and the risk of developing a PBC or CBC at the highest was 11% over the same time period, our data suggest that intensive breast cancer surveillance strategies for the BRCA-associated ovarian cancer group might be reconsidered, while risk reducing mastectomy is not indicated unless in specific cases requiring careful consideration in a multidisciplinary setting. Further studies in larger groups are warranted.

P4-11-05
Association between Bisphosphonate Use in Metastatic Breast Cancer (MBC) and Overall Survival.
Mathew A, Mathew IE, Rosenzweig MQ, Brufskey AM. University of Pittsburgh Medical Center; University of Pittsburgh Cancer Institute; Magee-Womens Hospital

Background: Pre-clinical studies on bisphosphonates in breast cancer have suggested an anti-tumor effect in addition to its bone protective role. However, randomized controlled trials of bisphosphonates versus placebo have found little evidence of increased overall survival (OS) in MBC. We conducted a retrospective single institution cohort study of MBC patients to evaluate the association between bisphosphonate use and overall survival.

Methods: Baseline demographic and tumor specific data were collected on newly diagnosed MBC patients between January 1998 and December 2009. Other variables included the number and sites of each metastasis, use of baseline neoadjuvant and adjuvant chemotherapy, and use of hormonal therapy. Bisphosphonate use was defined as present if it was administered for a period of at least 3 months in the metastatic setting. Overall survival was determined from the date of diagnosis of first metastatic disease. Survival analysis was performed using the Kaplan-Meier method and Cox proportional-hazards model.

Results: Data were available on 737 patients with MBC, of whom 434 died during a median follow-up of 2 years; median age was 50.3 years. 92% of patients were Caucasian; 32% were both ER and PR-negative; and 31% were HER2-positive. Over 67% of MBC patients had bone metastasis and nearly 80% received bisphosphonates. Multivariate analysis found an overall survival benefit for bisphosphonate use, with a hazard ratio of 0.63 (95% confidence interval: 0.48-0.84; p=0.002), when adjusted for variables with significant effect on survival on univariate analysis and other known prognostic variables. These variables include age, stage at diagnosis, race, hormone receptor status, HER2 status, and number of metastatic sites, presence of bone metastasis and the use of adjuvant therapy. The administration of adjuvant therapy did not yield a significant survival advantage in the analyses.

Conclusion: This retrospective cohort study provides evidence for an OS benefit with the use of bisphosphonates in MBC even after controlling for other significant prognostic factors.

P4-11-06
Uptake of Selective Estrogen Receptor Modulators and Other Breast Cancer Prevention Strategies among High-Risk Women Seen in a Breast Center.

Background: Selective estrogen receptor modulators (SERMs), tamoxifen and raloxifene, are FDA-approved for breast cancer (BC) risk reduction. However, uptake has been poor in the prevention setting, partly due to a lack of knowledge in the medical community about BC prevention and public misconceptions about the risks of SERMs. We assessed demographic and clinical factors that influence SERM uptake among high-risk women seen in an academic breast center, where specialized risk counseling is provided by a breast surgeon or medical oncologist.

Methods: Potential subjects included high-risk women seen for an initial consultation by Breast Surgery or Medical Oncology. Eligibility for SERM use included a 5-year Gail risk ≥1.67%, lobular carcinoma in situ (LCIS), BRCA mutation carrier, or estrogen receptor (ER)-positive and/or progesterone receptor (PR)-positive ductal carcinoma in situ (DCIS). Demographic and BC risk factor data was collected from self-administered questionnaires. Clinical data, including prior/current SERM use, was abstracted from medical chart review. Differences in distribution of risk factors, between women who ever took a SERM and those who did not, were examined using chi-square statistics or Fisher’s exact test. Multivariable logistic regression models were used to calculate odds ratios (OR) and 95% confidence intervals using SERM use as the dependent variable.

Results: Among 247 high-risk women enrolled between March 2007 and January 2011, median age 51 (17-82); White/Hispanic/Black/Asian (%): 55/32/7/6. 85% of women were undergoing annual mammography; 94% had a breast biopsy, 19% genetic testing, and 71% Medical Oncology referral. Among 181 (73%) women eligible for a SERM, Gail risk ≥1.67%/LCIS/DCIS/BRCA mutation (%): 35/22/39/3; 83 (46%) ever took a SERM, including 62 on tamoxifen and 21 on raloxifene. Early SERM discontinuation was only 7%. In multivariable analysis, significant predictors of SERM uptake included risk category (DCIS vs. Gail risk ≥1.67%/LCIS/BRCA mutation), higher income, higher body mass index (BMI), and referral to Medical Oncology. In terms of this high-risk population meeting American Cancer Society (ACS) behavioral guidelines for cancer prevention, 53% had a BMI <25 kg/m2, 44% consumed ≤1 alcoholic beverage per day, and 10% engaged in ≥4 hours of moderate physical activity per week; only 3.5% met all 3 recommendations.

Conclusions: Among high-risk women seen at a specialized breast center, application of clinical recommendations such as screening mammography, genetic testing, and SERM uptake were relatively high, suggesting that a comprehensive approach to the management of high-risk women is feasible. However, meeting ACS nutrition and physical activity guidelines for cancer prevention was limited, perhaps due to a lack of reimbursable staff to implement these guidelines. Breast cancer risk assessment and available interventions for prevention among high-risk women are underutilized in the U.S. Future studies should focus on the development and delivery of breast cancer prevention strategies.
P4-11-07


Background: Currently there are no real attempts internationally to tailor breast screening programmes to individual risk.

Methods: We have assessed the feasibility of collecting breast cancer risk information during routine mammographic screening in the National Health Service Breast Screening Programme (NHSBSP) in England, in order to consider, ultimately, adapting the screening interval to risk of breast cancer and introducing preventive strategies in women at high risk. The study Predicting Risk Of Cancer At Screening (PROCAS) aims to recruit 60,000 women over 3 years. Results: 26,000 women (June 8th 2011) have so far given consent to join the study. Thirty six percent of the first 20,000 women in nineteen screening sites in Manchester consented to enter the study and completed a risk factor questionnaire. The median 10 year breast cancer risk was 2.65%, with 926 (9.26%) of the first 10,000 women having a 10 year risk of ≥5% and 92 (0.92%) having a 10 year risk of ≥8% (Tyrer-Cuzick), IQR:1.35. 832 (8.32%) women had a mammographic density of 60% or greater (Visual Analogue Scale). We collected saliva samples from 1019 women for genetic analysis and will extend this to 18% of participants. Of those who agreed to participate in the study, 94% indicated that they wished to know their breast cancer risk. Women with a 10-year risk of ≥8%, and women with a 10-year risk of ≥5% and mammographic density ≥60% were invited to attend or be telephoned to be counselled. To date 138 have accepted with 135, so far, having received risk counselling. Nineteen percent of the high-risk women identified subsequently decided to enter a randomised breast cancer prevention study with either a dietary or drug intervention (IBIS2, anastrozole vs placebo). Results from the first 1,000 women who provided DNA samples suggest that the risk information from the 18 validated SNPs may enhance existing risk models. Conclusion: This study demonstrates that it is feasible to determine individual breast cancer risk and offer women appropriate risk-reducing interventions within the context of a population-based mammographic screening programme.

P4-11-08
Changes in the Distribution of Loco-Regional and Distant Breast Cancer Recurrences over the Last 20 Years: Implications for Patient Care and Future Research.

Bouganim N, Clemons M, Amir E. The Ottawa Hospital Cancer Center, Ottawa, ON, Canada; Princess Margaret Hospital, Toronto, ON, Canada

Introduction: Improvements in adjuvant therapy have led to a sustained fall in recurrences after early breast cancer. The differential reduction of both local-regional and systemic recurrences is poorly understood. This study aimed to explore changes in the distribution of loco-regional and distant recurrences in clinical trials reported over the last 20 years. We also aimed to determine the relative impact of adjuvant chemotherapy and endocrine therapy.

Methods: A MEDLINE search for adjuvant, Phase III randomized breast cancer clinical trials between January 1990 and March 2011 was performed. Neo-adjuvant, single agent biologics and studies that did not report the proportion of loco-regional and distant recurrences were excluded. Change in the frequency of recurrences was assessed as the non-parametric correlation between the number of loco-regional recurrences (as a proportion of all recurrences) and time. Studies were weighted by sample size. Pre-specified subgroup analyses were assessed using the interaction test and included type of surgery performed, radiotherapy use, menopausal status and type of systemic therapy delivered. Definition of local and distant recurrences differed between studies. For consistency, loco-regional recurrences were classified as recurrences limited to the ipsilateral breast, chest wall, axillary, supraclavicular and internal mammary lymph nodes. Any other recurrence was defined as distant, with the exception of contralateral breast cancer, that was excluded from this analysis.

Results: Fifty-three randomized clinical trials with a total of 86,598 patients were included in the analysis. Between 1990 and 2011, the proportion of loco-regional recurrences has decreased from approximately 50% to 10% (Spearman’s rho = -0.40, p<0.001).

Conclusion: Advances in treatment of early breast cancer have differentially reduced the proportion of loco-regional recurrences compared with distant recurrences. In recent trials, loco-regional recurrences account for less than 10-15% of all recurrences. These falling event rates may affect patient care, especially when deciding on treatments influencing loco-regional control. This change may also impact on the design of clinical trials assessing loco-regional therapy such as surgery and/or local radiation therapy.

P4-11-09
Polymorphisms Related to Steroid Hormone Concentrations in Nipple Aspirate Fluid (NAF).

Lee O, Chatterton RT, Shidfar A, Wang J, Scholten D, Khan SA. Northwestern University, Chicago, IL

Background: The steroid hormone concentrations in NAF are variable, and differ significantly from systemic levels. Single nucleotide polymorphisms (SNPs) in genes associated with the metabolism of estradiol (E2) to 4-hydroxyestradiol (CYP1B1) and a transporter of steroid sulfate uptake (SLCO2B1) may partially determine the steroid hormone level in NAF, and thereby contribute to breast cancer risk. We determined the relationship between SNPs of interest and the measured concentrations of sex steroids in NAF.

Methods: Blood samples of 263 women at high risk of breast cancer who produced NAF were extracted for gDNA, and 40 ng of gDNA was used to determine the presence of the selected SNPs or their wild type genes in all subjects by the Taqman Drug Metabolism panel. The concentrations of six steroid hormones, estradiol(E2), estrone (E1), progesterone (P4), testosterone (T), androstenedione (A4), and dehydroepiandrosterone (DHEA), from NAF were measured by immunoassay procedures after extraction and purification by High Pressure Liquid Chromatography. Comparisons of NAF steroid hormone concentrations were made for the polymorphism and wild type groups using the nonparametric Mann-Whitney tests (significance p < 0.05, two-tailed).
Results: 46% of the subjects carried the V432K polymorphism of CYP1B1 (rs1056836, C>G), and 45% of the subjects carried the S486F polymorphism of SLCO2B1 (rs2851069, C>T). All NAF hormone concentrations are represented as a median with a quartile range (25%, 75%). The NAF P4 levels of the V432K mutation carriers of CYP1B1 were significantly lower than those of subjects with the wild type alleles: 3.54 (1.62, 8.79) ng/mL and 7.65 (2.13, 27.77) ng/mL, respectively (p = 0.002). Those with the S486F polymorphism of SLCO2B1 had significantly higher E1 levels than the wild type subjects: 0.35 (0.14, 1.12) ng/mL and 0.25 (0.11, 0.59) ng/mL, respectively (p = 0.026) and significantly lower P4 levels: 3.77 (1.71, 11.53) ng/mL and 6.65 (2.34, 22.24) ng/mL, respectively (p = 0.021). In addition, we found that the women carrying both the V432K and S486F mutations had significantly lower P4 levels than the women carrying only the S486F mutation: 2.43 (1.45, 6.67) ng/mL and 6.94 (2.13, 26.64) ng/mL (p = 0.013). No associations were found between the other hormones and these polymorphisms.

Conclusions: The anticipated effects of CYP1B1 in increasing clearance of E2 and E1 were not observed. Instead, NAF E1 concentrations were significantly increased. In contrast, with the S486F polymorphism of SLCO2B1 the expected decrease in E1 and E2 in NAF was not observed; nor was the DHEA concentration decreased. These expectations were based on the reported lower activity of the S486F gene product. The decreased concentrations of NAF P4 associated with both polymorphisms are difficult to explain. Additional studies are required to understand the observed associations, but these findings raise the possibility that low P4 levels in NAF may be genetically determined, and suggest the hypothesis that this polymorphism may be related to a decreased risk of breast cancer.

P4-11-10
Perceptions, Knowledge and Satisfaction with Contralateral Prophylactic Mastectomy among Young Women with Breast Cancer.
Tracy MS, Meyer ME, Sepucha K, Gelber S, Hirshfield-Bartek J, Troyan S, Morrow M, Schapira L, Come S, Winer E, Partridge AE, Dana-Farber Cancer Institute, Boston, MA; Massachusetts General Hospital, Boston, MA; Memorial Sloan-Kettering Cancer Center, New York, NY; Beth Isreal Deaconess Medical Center, Boston, MA

Background: There has been an increasing prevalence of contralateral prophylactic mastectomy (CPM), particularly among younger women with breast cancer. There has been limited research evaluating patient preference, knowledge and decision-making regarding this issue. Methods: We surveyed women who had bilateral mastectomy who were enrolled in a multicenter, longitudinal cohort study of women diagnosed with breast cancer at age 40 and younger. The CPM survey included 23 items on decision making, knowledge, and satisfaction with CPM. Results: Of the 550 patients enrolled as of November 2010, 157 (28.5%) had bilateral mastectomy, of whom 124 completed the CPM survey (response rate 79%). Women with bilateral breast cancer (3% or bilateral prophylactic (1) indications for surgery were excluded. Median age at diagnosis was 37 years (range 26-40); 26 women (21%) reported having a genetic mutation (21 BRCA1 and 5 BRCA2). Excluding mutation carriers, women estimated that a median of 10 of 100 women (range 0-90) would develop contralateral breast cancer in the 5 years after unilateral breast cancer treatment and that 5 of 100 women (range 0-98) treated with CPM would develop contralateral breast cancer. Eighteen percent of all respondents believed that women who undergo bilateral mastectomy live longer. Women were asked the importance of potential reasons for undergoing CPM (see Table 1). Eighty-two percent of women were "extremely confident" in their decision to undergo CPM and 92% would "definitely" still choose CPM.

Table 1: Importance of reasons for choosing CPM

<table>
<thead>
<tr>
<th>Importance</th>
<th>Extremely/Very Important</th>
<th>Somewhat Important</th>
<th>Not at all Important</th>
<th>Not Sure</th>
<th>Missing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extensive chance of CBC</td>
<td>117 (84)</td>
<td>3 (2)</td>
<td>1 (1)</td>
<td>1 (1)</td>
<td></td>
</tr>
<tr>
<td>Peace of mind</td>
<td>113 (81)</td>
<td>4 (3)</td>
<td>1 (1)</td>
<td>1 (1)</td>
<td></td>
</tr>
<tr>
<td>Extend life</td>
<td>111 (79)</td>
<td>3 (2)</td>
<td>6 (5)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Feeling at increased risk of CBC</td>
<td>106 (75)</td>
<td>7 (5)</td>
<td>5 (4)</td>
<td>1 (1)</td>
<td></td>
</tr>
<tr>
<td>Prevent metastasis</td>
<td>103 (74)</td>
<td>8 (6)</td>
<td>11 (9)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Cosmetic symmetry</td>
<td>21 (15)</td>
<td>2 (2)</td>
<td>17 (14)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Worry that screening wouldn’t work</td>
<td>61 (43)</td>
<td>10 (8)</td>
<td>26 (22)</td>
<td>3 (3)</td>
<td></td>
</tr>
<tr>
<td>Strong family history</td>
<td>44 (31)</td>
<td>10 (8)</td>
<td>57 (47)</td>
<td>2 (2)</td>
<td></td>
</tr>
<tr>
<td>Follow doctor recommendation</td>
<td>40 (29)</td>
<td>23 (18)</td>
<td>55 (44)</td>
<td>4 (3)</td>
<td></td>
</tr>
<tr>
<td>Cosmetic improvements</td>
<td>35 (25)</td>
<td>29 (22)</td>
<td>55 (44)</td>
<td>4 (3)</td>
<td></td>
</tr>
<tr>
<td>Known genetic mutation</td>
<td>32 (22)</td>
<td>2 (2)</td>
<td>72 (56)</td>
<td>4 (3)</td>
<td></td>
</tr>
<tr>
<td>Abnormal mammogram before surgery</td>
<td>13 (9)</td>
<td>6 (5)</td>
<td>82 (68)</td>
<td>5 (4)</td>
<td></td>
</tr>
<tr>
<td>Advice from family/friends</td>
<td>17 (12)</td>
<td>30 (23)</td>
<td>60 (46)</td>
<td>7 (6)</td>
<td></td>
</tr>
<tr>
<td>Abnormal MRI before surgery</td>
<td>12 (10)</td>
<td>3 (3)</td>
<td>88 (73)</td>
<td>6 (5)</td>
<td></td>
</tr>
<tr>
<td>Abnormal ultrasound before surgery</td>
<td>11 (9)</td>
<td>4 (3)</td>
<td>90 (73)</td>
<td>6 (5)</td>
<td></td>
</tr>
<tr>
<td>Cancer in other breast before surgery</td>
<td>10 (8)</td>
<td>1 (1)</td>
<td>94 (78)</td>
<td>5 (4)</td>
<td></td>
</tr>
<tr>
<td>Prior radiation to chest</td>
<td>5 (4)</td>
<td>4 (3)</td>
<td>95 (79)</td>
<td>7 (6)</td>
<td></td>
</tr>
</tbody>
</table>

Conclusion: Young women with breast cancer have high rates of CPM. Many young women who have undergone CPM overestimate the risk of contralateral disease and the benefits of CPM, including believing that CPM will prevent metastasis and extend life. Interventions to counsel young women with early breast cancer to help them make optimal surgical treatment decisions are needed.

P4-11-11
Thyroid Disease Is Associated with Breast Cancer: A Meta-Analysis.
Hardefeldt PJ, Esliek GD, Edirimanne S. The University of Sydney, Sydney, NSW, Australia; Nepean Hospital, Penrith, NSW, Australia

Background: The controversial relationship between benign thyroid diseases and breast cancer has been investigated for over 50 years. Despite extensive population studies, the results as a whole have been inconsistent. The purpose of this study was to collate and analyse available data, calculating a pooled odds ratio of the risk of breast cancer in patients diagnosed with benign thyroid diseases.

Materials and Methods: Studies were obtained from a database search of MEDLINE, EMBASE, PubMed, Current Contents and Google Scholar with additional cross checking of reference lists. Inclusion criteria required a confirmed diagnosis of a benign thyroid disease, reporting of an odds ratio or data to calculate an odds ratio (and 95% confidence interval) and the use of an internal control group as the comparator. Collated data was assessed for heterogeneity and a pooled odds ratio calculated. From 276 citations identified in this search, a total of 28 studies were identified meeting our inclusion criteria. No language restrictions were used in the search or study selection. All data were analysed using a random effects model.

Results: There was significant evidence of an increased risk of breast cancer in patients with auto-immune thyroiditis, evident in a pooled odds ratio (OR) of 2.92 (95% CI: 2.13-4.01). No heterogeneity was present (I²=0, p=0.62) and there was no publication bias (p=0.15). In addition, the results supported an increased risk associated with the presence of anti-thyroid antibodies (OR 2.02, 95% CI: 1.63-2.50) and goitre (OR 2.19, 95% CI: 1.44-3.33). Moderate but non-significant heterogeneity was present in the analysis of goitre (I²=49.2, p=0.08). No heterogeneity was present in the analysis of antibody presence, moderate and non-significant heterogeneity was evident (I²=58.3, p=0.08) with no publication bias (p=0.07). Subgroup analysis of antibody presence revealed increased risk associated with both anti-TPO (OR 2.64, 95% CI: 1.82-3.83)
and anti-Tg antibodies (2.71, 95% CI: 1.58-4.69). No heterogeneity was present in the analysis of either subgroup evidenced in I² index of 29.3 (p=0.22) and 18.1 (p=0.30), respectively. Publication bias was not significant (p=0.53 and 0.17). Quantitative analysis of hypothyroidism and hyperthyroidism was not significant, evident in pooled odds ratios of 1.56 (95% CI: 0.78-3.12) and 1.56 (95% CI: 0.84-2.88), respectively. Heterogeneity was high in hypothyroidism (I²=80.9, p=0.001) yet minimal in hyperthyroidism (I²=0, p=0.75). Publication bias was not significant in either hypothyroidism (p=0.06) or hyperthyroidism (p=0.20).

Conclusion: While these results indicate a link between thyroid autoimmunity and breast cancer, further prospective studies are required to definitively prove causality.

### P4-11-12

**Molecular Phenotype of Breast Cancers in a Large Cohort of Young Women According to Time Interval Since Pregnancy.**

Collins LC, Gelber S, Marotti JD, Cole K, Kereakoglow S, Ruddy KJ, Brachtel EF, Schapira L, Come SE, Borges VF, Schedin PJ, Warner E, Winer E, Partridge A. Beth Israel Deaconess Medical Center, Boston; Harvard Medical School, Boston; Dana Farber Cancer Institute, Boston; Dartmouth-Hitchcock Medical Center, Lebanon, Hanover, NH; Brigham and Women’s Hospital, Boston; Massachusetts General Hospital, Boston; University of Colorado Cancer Center, CO; Sunnybrook Odette Cancer Centre, Toronto, ON, Canada

**Background:** The increase in breast cancer risk during pregnancy and post partum is well recognized. The cross-over to protective effect does not occur until many years later and varies with age at first birth. Recently, a genomic signature specific to the pregnant compared with the non-pregnant breast has been identified; this signature remains present in the postmenopausal parous breast. Given this, we investigated whether time interval since pregnancy affects the phenotype of breast cancers arising in young women compared with nulliparous women. Methods: We examined molecular phenotype, according to histologic grade and biomarker status, in relation to time since pregnancy in an ongoing prospective cohort study (n=355) of young women (≤40yrs) with breast cancer. Medical records were reviewed for tumor stage and receptor status. Parity was ascertained from questionnaires completed within 9 months of diagnosis. Tumor grade was determined by central pathology review. Using tumor grade and biomarker expression, cancers were categorized as luminal A (ER+ and/or PR+, HER2-, histologic grade 1 or 2); luminal B (ER+ and/or PR+, HER2+, or ER and/or PR+, HER2- and grade 3); HER2 type (ER-, PR-, HER2+); and triple negative (ER-, PR-, HER2- and grade 3); HER2 type (ER-, PR-, HER2+); and triple negative (ER-, PR-, HER2-). Results: The median age of the study population is 37 years (range 17-40). Overall, 80% of women had stage 1 or 2 disease; 67% of cancers were ER positive and 32% showed HER2 overexpression. The distribution of breast cancer molecular phenotypes by time interval since last pregnancy is shown in the table.

<table>
<thead>
<tr>
<th>Molecular Phenotype</th>
<th>Nulliparous N=129 (36%)</th>
<th>≤2 years N=51 (14%)</th>
<th>&gt;2-5 years N=78 (22%)</th>
<th>≥5 years N=97 (27%)</th>
<th>Total patients N=355</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luminal A (ER+, PR+, HER2-, grade 1 or 2)</td>
<td>52 (40)</td>
<td>13 (25)</td>
<td>20 (26)</td>
<td>33 (34)</td>
<td>118 (33)</td>
</tr>
<tr>
<td>Luminal B (ER+, PR+, HER2+, or ER and/or PR+, HER2- and grade 3)</td>
<td>46 (36)</td>
<td>20 (39)</td>
<td>27 (35)</td>
<td>33 (34)</td>
<td>126 (35)</td>
</tr>
<tr>
<td>HER2 type (ER-, PR-, HER2+)</td>
<td>9 (7)</td>
<td>6 (12)</td>
<td>14 (18)</td>
<td>9 (9)</td>
<td>38 (11)</td>
</tr>
<tr>
<td>Triple negative</td>
<td>22 (17)</td>
<td>12 (24)</td>
<td>17 (22)</td>
<td>22 (23)</td>
<td>73 (21)</td>
</tr>
<tr>
<td>Tumor grade 3</td>
<td>66 (23)</td>
<td>15 (23)</td>
<td>64 (29)</td>
<td>50 (21)</td>
<td>194 (55)</td>
</tr>
</tbody>
</table>

**In our large cohort of parous young women, we found no differences in the distribution of molecular phenotype according to time interval since pregnancy. However, nulliparous young women were more likely to develop luminal A cancers compared to parous women. (40% vs. 29%; unadjusted chi square p-value=0.03) and appeared less likely to develop HER2-type and triple negative cancers (7% vs. 13%, p-value=0.09 and 17% vs. 23%, p-value=0.22 respectively). There were no differences in the distribution of luminal B cancers. Conclusions: The distribution of molecular phenotypes is similar among parous young women regardless of the time interval since parturition. Nulliparous young women appear more likely to develop luminal A cancers compared to parous women. Whether the difference in molecular phenotypes of pregnancy-associated breast cancers vs. cancers arising in nulliparous women is due to the effects of genomic alteration remains to be investigated. Effects of a prior pregnancy appear consistent across a 5-year period, in keeping with the concept of genomic alterations identified in the normal pregnant breast and thereafter.**

### P4-11-13

**Influence of Two Years of Exemestane on Bone Mineral Density in Postmenopausal Women at Increased Risk of Developing Breast Cancer: a Companion Study to the NCIC CTG MAP3 Trial.**

Goss PE, Richardson H, Ingle JN, Chlebowski RT, Fabian CJ, Garber JE, Sarto GE, Hiltz A, Tu D, Cheung AM. Massachusetts General Hospital Cancer Center, Boston, MA; Queen’s University, Kingston, ON, Canada; Mayo Clinic, Rochester, MN; Los Angeles Biomedical Research Institute, Torrance, CA; University of Kansas Medical Center, Westwood, KS; Dana Farber Cancer Institute, Boston, MA; Center for Women’s Health and Health Research, Madison, WI; General Hospital, Toronto, ON, Canada

**Background:** Exemestane significantly reduced invasive and pre-invasive breast cancers in postmenopausal women at increased risk for breast cancer in the NCIC CTG MAP3 trial with no serious toxicities, including excess fractures or osteoporosis. **Purpose:** To provide additional information on the effect of exemestane on bone loss in women at high risk for breast cancer, within a subset of women participating on the NCIC CTG MAP3 trial. The primary hypothesis is that exemestane does not induce clinically significant bone loss in postmenopausal women at increased risk of developing breast cancer at 2 years. The primary objective of this companion study is to examine the effect of exemestane on lumbar spine and total hip BMD by DEXA at 2 years in women participating in the MAP3 trial.

**Methods:** The MAP.3B bone sub-study registered women from the main MAP.3 trial from May 2008 to March 2010. Eligible women had to have an acceptable quality BMD scan by DEXA taken within 12 months prior to randomization to MAP3. A BMD T-score >-2.0 SD (i.e. better than 2 standard deviations below the average peak BMD of a young adult woman) was established as the study population cutoff. A questionnaire including information on height, falls, fractures, lifestyle information including physical activity, tobacco and alcohol use was completed at baseline, 12 months, 24 months and at last visit. Fasting serum for bone biomarkers was collected at 12 months and total hip and L1-L4 (postero-anterior) spine BMD were measured 2 years after randomization on the same Lunar or Hologic scanner. The primary objective was to determine differences in hip and spine BMD at 2 years. Secondary outcomes include number of skeletal fractures and development of osteoporosis 2 years after randomization and changes in bone biomarkers at 1 year after randomization. For the analysis of the primary endpoints, the upper limit of a one-sided 95% confidence interval for the difference in mean percentage changes between placebo and exemestane will be calculated for the BMD by DEXA at each site. We will conclude that exemestane does not induce significant bone loss in postmenopausal women at increased risk of...
developing breast cancer at 2 years when the upper limit is less than 3% for both sites. Similar confidence interval approach will be used to analyze the secondary endpoints.

**Results:** Between May 2008 and March 2010, 238 postmenopausal women were recruited. Median age was 61.8 years, and the majority of women were Caucasian (91%), with approximately 20% of the participants reporting a recent fall (within past 12 months) and another 13% reporting a recent fracture prior to randomization. We will report results from the primary as well as the secondary endpoints at the SABCS meeting.

**P4-11-14**

**Number Needed To Treat (NNT) as a Measure of Incremental Drug Benefit: Denosumab vs. Zoledronic Acid for the Prevention of Skeletal Related Events (SREs) in Advanced Breast Cancer.**

**Dranitsaris G, Kaura S. Augmentium Pharma Consulting, Toronto, Canada; Novartis Pharmaceuticals, NJ**

**Background:** Intravenous zoledronic acid (ZOL) is the standard of care for the prevention of SREs in advanced breast cancer. However, monthly subcutaneous denosumab (Dmab) was recently approved in the US, as an alternative to ZOL based on the results of a large randomized trial which demonstrated a prolongation in median time to first SRE (HR = 0.82, p = 0.01) and a 5.8% (p = 0.01) absolute reduction in SREs in favour of Dmab over the 34 month study period. The challenge for clinicians and payers is how to reconcile the modest benefits of Dmab with the cost, which is approximately twice that of ZOL. NNT represents the number of patients that need to be treated with a new intervention in order to avoid one additional event, and is a widely accepted approach used to make sense of numerical results from clinical trials. In this analysis, the NNT approach was used to assess the incremental benefit of Dmab over ZOL for the prevention of SREs in advanced breast cancer.

**Methods:** The pivotal randomized trial for Dmab vs. ZOL in breast cancer (Stopeck, JCO 2010 & US PI 2010) was reviewed. As an alternative to ZOL, the NNT with Dmab to avoid any SRE at 12 and 34 months (trial end) was determined. NNT by type of SRE was also estimated. These consisted of pathologic fractures, radiation to bone, spinal cord compressions and surgery to bone. The calculated NNT represents the number of patients that need to be treated with a new intervention in order to avoid one additional event, and is a widely accepted approach used to make sense of numerical results from clinical trials. In this analysis, the NNT approach was used to assess the incremental benefit of Dmab over ZOL for the prevention of SREs in advanced breast cancer.

**Results:** To avoid a single SRE after 12 months of treatment of continuous therapy with Dmab, approximately 36 patients need to be treated. To avoid a single fracture and radiation to bone, approximately 39 and 27 patients need to be treated with Dmab over a 34 month period. Dmab was unable to offer any incremental benefit over ZOL in terms of avoiding spinal cord compressions or surgery to bone after 34 months of treatment.

**Discussion:** The NNT approach is a simple and effective method to express the findings in a clinically meaningful way. In this analysis, the incremental benefit of Dmab would only be realized when a minimum of 36 patients are treated for 12 months. For the more severe skeletal-related events, in terms of clinical and economic burden to patients and society, such as spinal cord compression and surgery to bone, Dmab did not offer any incremental benefit over ZOL in terms of SRE avoidance. This marginal incremental benefit needs to be considered alongside the high cost of Dmab.

**P4-11-15**

**Increased Propability of Triple Negative Breast Cancer (TNBC) in Premenopausal Patients after Exogenous Hormonal Intake (EHI).**

**Pistamaltzian NF, Tzavara C, Papadimitriou C, Gyftaki R, Tryfonopoulou D, Papanoulous C, Tsoukalas N, Koumakis G, Demiri S, Koutopoulou N, Misitiz Y, Apostolikas N, Efremidis A. Agios Savvas Cancer Hospital of Athens, Athens, Greece; University of Athens, Athens, Greece**

**Purpose:** HRT is a known risk factor for breast cancer (BC) among post-menopausal women. Our knowledge in relation to EHI (estrogens and/or progesteron) and BC among pre-menopausal women is scarce. We have studied whether previous EHI influences BC phenotype, clinical and pathologic characteristics and correlation with other known BC risk factors.

**Patients and methods:** A prospective analysis (data recorded upon patients first presentation) of an electronic database at a tertiary cancer centre was performed. Patients’ demographics, risk factors for BC (smoking, alcohol use, obesity, family history), clinical profile, EHI parameters (duration, cause) and the tumors’ histopathology (type, grade,ER/PgR and HER2 by IHC and FISH) were analyzed.

Premenopausal patients without an EHI history consisted control group.

**Results:** Out of 938 patients treated for BC between 2006 and 2010, 333(35,5%) were premenopausal and 131(39%) of them reported any use of hormones. Median age was identical (43 years,range:20-57) among premenopausal patients with and without EHI history. Mean duration of use was 28 months (range:1-180). Causes of EHI were contraception (35%), pregnancy(17%), menstrual abnormalities(17%) and medically assisted fertilization(17%). Smoking, alcohol use and obesity didn’t differ among two groups. Family history for BC was more common (31, 3% vs. 22,8%, p=0,08)among women with EHI. No correlation was found during duration of use and the time of cancer diagnosis, while the mean time from the cessation of hormones to cancer diagnosis was 13 years(range:1-32). Only 18% of breast cancers were diagnosed within the first 5 years after exogenous hormones cessation.

TNBC was found to be significantly increased among premenopausal women with a history for EHI (23,6% vs. 13,4%, p=0,016). This increase was independent of the existence of positive family history for BC (p=0,61). EHI conferred a twofold increase in the risk for a TNBC (OR=1,99 p=0,019). No other clinical or histopathologic parameter showed any difference among the two groups.

**Conclusion:** Prior use of exogenous hormones, for any cause and irrespective of the coexistence of other risk factors and family history, increases the probability of a triple negative breast cancer diagnosis by twofold. Whether this represents a trend of a changing epidemiology in the types of BC in prior hormone users vs. non-users, poses an extremely challenging hypothesis to be verified in large epidemiologic studies - given the young age and the treating difficulties of this patient population.

**P4-11-16**

Withdrawn by Author

**P4-11-17**

**Noninferiority (NI) Phase III Trials in Advanced Breast Cancer (ABC) over 12 Years.**

**Saad ED, Militao MS. Dendrix Research, Sao Paulo, Brazil**

**Background:** NI trials have gained increasing importance in oncology. The contemporary practice regarding the design features
and the results of NI trials have not been formally assessed in ABC.


**Results:** We retrieved 93 reports, 12 of which (13%; 95% CI, 7% – 22%) had a NI primary hypothesis. Of note, there were no equivalence (i.e., two-sided question) studies. There was no significant trend in the proportion of NI trials in the two 6-year periods compared (17% vs 9%; P=0.36). The median (range) sample sizes for NI and superiority trials were 404 (160-1354) and 326 (118-907) patients, respectively (P=0.32). NI trials enrolled exclusively first-line patients more frequently than superiority trials (83% vs 51%; P=0.06). The primary endpoint for NI trials was response rate (RR) and progression-free survival/time to tumor progression in 8 and 4 cases, respectively. Despite their nominally higher number of patients, NI trials were more likely than superiority trials to have RR as their primary endpoints (67% vs 32%; P=0.03). The NI margin was reported in all trials, but justified by authors in only one case. Among trials with RR as primary endpoint, the NI margin was 15% in six cases and 25% in two. In trials with progression-free survival/time to tumor progression as primary endpoints, the lower limit of the confidence interval for the hazard ratio to demonstrate NI was always 0.8. Of 12 NI trials, 9 were positive (i.e., proved NI).

**Conclusion:** NI trials represent 13% of contemporary phase III trials on ABC. Most such trials achieve their primary endpoint. At present, NI margins seem to be chosen on historical basis rather than on statistical reasoning, and such choice could bear implications on trial positivity.

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**P4-11-18**

**Mammographic Surveillance in Atypical Hyperplasia of the Breast and Subsequent Development of Cancer: A Need for Long Term Follow Up.**

Koron R, Sridharan U, Mitchell G, Holcombe C. Royal Liverpool and Broadgreen University Hospital Trust, Liverpool, Merseyside, United Kingdom

**Introduction**

Atypical hyperplasia of the breast is a known risk factor for breast cancer. Despite this risk there are no published guidelines on a recommended follow up regime for these patients.

**Methods**

A retrospective study was carried out on 1920 core biopsy results within a major screening breast unit from 2001 to 2005. Patients who had a final diagnosis of Atypical Ductal Hyperplasia (ADH), Atypical Lobular Hyperplasia (ALH) and Lobular Carcinoma in Situ (LCIS) were included.

Information was extracted from multidisciplinary team meeting records, departmental data bases, pathology and radiology reports. Details of follow up and subsequent cancer diagnosis were noted.

**Results**

83 patients were diagnosed with ALH, ADH or LCIS from a core biopsy. The mean age was 54 years (range 40-85 years). Seventy six (91.6%) of these patients went on to have an excision biopsy. Sixty four (77.1%) women have received long term mammographic follow up ranging from 5 to 9 years to date. Nineteen patients did not receive mammographic surveillance. Of this group 2 patients with LCIS had bilateral risk reducing mastectomies and 2 patients died shortly after diagnosis with no evidence of breast cancer. Eight patients developed breast cancer 3 to 8 years after their initial diagnosis. Of these, four patients developed cancer in the same breast that atypia had been diagnosed in previously, and four in the contra lateral breast.

**Conclusion**

12.5% of patients in the follow up group developed breast cancer within 8 years of diagnosis of atypical hyperplasia. A review of the literature suggests that in view of the increased risk of subsequent breast cancer in this group of women long term follow up is required. On the basis of these findings we recommend that all patients diagnosed with ALH, ADH or LCIS should be entered into an 18 monthly mammogram surveillance programme for 15 years.

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**P4-11-19**

**Healthcare Resource Utilization among Breast Cancer Patients with Bone Metastases and Skeletal-Related Events: A Population-Based Cohort Study in Denmark (1997 - 2009).**

Yong M, Christiansen CF, Gammelager H, Sverke C, Chia V, Atchison C, Fryzek J. Amgen Inc, Thousand Oaks, CA; Aarhus University Hospital, Aarhus, Denmark; Exponent, Alexandria, VA

**Background:** The healthcare resource utilization (HRU) of breast cancer patients who develop bone metastases and skeletal-related events (SREs) has not been well-characterized. Our objective was to describe the HRU associated with SREs in a large population-based cohort of Danish breast cancer patients with bone metastases and one or more SRE.

**Methods:** We identified women diagnosed with incident breast cancer from January 1, 1997 through December 31, 2006 using the Danish Cancer Registry. We followed this cohort of patients for development of subsequent bone metastases and SREs identified through the Danish National Registry of Patients through December 31, 2009. SREs were defined as pathologic fracture, spinal cord compression, and radiation or surgery to bone. Among patients with only one SRE, the HRU period, composed of all HRU occurring within a 90-day period after the SRE and within a two-week diagnostic period prior to the SRE, was assessed. For patients with multiple SREs (each one separated by less than 90 days), the HRU period, composed of all HRU occurring within a two-week diagnostic period prior to the first SRE up until 90 days after the last SRE, was described. Patients may have had multiple HRU periods if SREs were separated by more than 90 days. The HRU summarized included number of inpatient hospitalizations, length of hospitalization stay, outpatient physician visits, emergency room visits, and procedures.

**Results:** We identified 1,148 patients with bone metastases and SREs among 38,485 breast cancer patients. The mean age at breast cancer diagnosis for those who developed bone metastases and SREs was 59 years (SD, 13 years) and the majority (72%) of patients had multiple SREs during the first HRU period. Approximately 20% to 30% of patients with single and multiple SREs, respectively, died within the first HRU period. Overall, length of hospitalization was longest for patients with spinal cord compression followed by patients with pathologic fracture. In general, patients with multiple SREs had higher HRU compared to those with a single SRE in the first HRU period, particularly in length of hospitalization stay.

**Conclusion:** SREs secondary to bone metastases are serious events. In Denmark, high HRU was observed in all patients with SREs, but especially in those with multiple SREs, where increased lengths of hospitalization were observed.
P4-11-20
Background: Obesity, post-diagnosis weight gain, and presence of metabolic syndrome in breast cancer are reported to adversely affect survival among breast cancer survivors. Most of the studies on weight gain and metabolic syndrome in breast cancer are from Western countries and few information is available on Asian population. We designed this prospective observational study to characterize weight and metabolic changes during adjuvant treatment in women with early breast cancer and to identify factors associated with occurrence of metabolic syndrome, focusing on dietary pattern.
Methods: Patients aged 18-75 who underwent curative surgery with stage I-III invasive breast cancer were enrolled from 2008 to 2010. We measured glucose (FBS), hemoglobin A1c (HbA1c), total cholesterol (TC), HDL cholesterol, and triglyceride (TG) level in fasting serum samples before starting adjuvant therapy, at 6 months and 12 months after enrollment. Body weight, body mass index (BMI), body fat mass, and percent body fat at baseline, 6 months, and 12 months were also measured. Dietary intake was assessed using valid semi-quantitative food frequency questionnaire (FFQ).
Results: Total of 63 patients were enrolled. Median age of the enrolled patients was 48 (range, 25-68), with premenopausal/postmenopausal 40 (63.5%)/23 (36.5%). Fifty (82.0%) and 10 (16.4%) received adjuvant chemotherapy followed by hormone therapy and hormone therapy alone. Hormone receptor positive (ER+/PR+) and HER2 positive cancer accounted for 52 (83.9%) and 7 (12.1%). Mean FBS, HbA1c, TC, HDL, and TG level was 99.9 mg/dL (range, 83-159), 5.59 mg/dL (range, 4.8-7.5), 197.4 mg/dL (125-298), 51.9 mg/dL (range, 30-90), and 119.7 mg/dL (42-371). Mean height, weight, and BMI was 158 cm (range, 149-169), 61.7 kg (range, 46.2-96.0), and 24.7 kg/m² (range, 18.7-35.7), respectively. According to the WHO and NIH guidelines for Asian, normal (BMI 18.5-22.9), overweight (BMI 23-24.9), and obesity (BMI≥25) was 18 (28.6%), 13 (20.6%), and 32 (50.8%), respectively. Number of patients with metabolic syndrome was 18 (34%). Mean BMI (26.1 vs 24.0, p=0.021) and TG (180.6 vs 92.0, p=0.001) was higher, HDL cholesterol was lower (42.2 vs 57.3, p<0.001) in patients with metabolic syndrome. Composition of daily calorie intake consisted of 13.5% (range 10.7-21.8) of protein, 6.7% (range, 3.3-22.1) of fat, and 70.1% (range, 28.1-79.5) of carbohydrate. The presence of metabolic syndrome was associated with a higher carbohydrate intake (carbohydrate intake per ideal body weight>6.0) (p=0.071). The TG level of patients who ingested high carbohydrate was significantly higher (143.8 vs 102.9, p=0.023). The HDL level of patients who took high fat diet (>20% of total calorie) was lower (45.3 vs 53.5, p=0.045).
Conclusion: In our cohort of Korean breast cancer patients, 34% had metabolic syndrome at baseline. Those patients with metabolic syndrome consumed higher proportion of carbohydrate, which resulted in significantly higher level of TG. Our data suggest that composition of calorie intake is different in Asian population compared to Western countries, warranting for reappraisal on the recommendation on life style modification and diet.

P4-11-21
A Retrospective Analysis of Women at Increased Lifetime Risk for Breast Cancer: Referral Patterns to Subspecialty Providers, Recommendations and Outcomes.
Background: Inheritance of an abnormal BRCA 1/2 gene, a family history of breast cancer (BrCa), or a personal history of lobular carcinoma in situ (LCIS), atypical hyperplasia, or chest wall radiation can significantly increase an individual’s lifetime risk for developing BrCa. In 2007, the American Cancer Society (ACS) released updated guidelines for screening in women with a lifetime risk of BrCa ≥20-25%. These guidelines added MRI screening to annual mammography. The objective of our analysis is to characterize patients referred after the release of the 2007 ACS guidelines to subspecialty providers specifically for evaluation of BrCa risk and analyze subsequent screening and risk reduction recommendations in the cohort of patients (pts) with a predicted increased lifetime risk for BrCa.
Methods: Pts seen at a single center (University of Wisconsin [UW]) between 1/2007-3/2011 by medical, surgical and/or gynecology-oncology for an increased lifetime risk of BrCa were identified by billing codes or evaluation in the UW Breast Cancer Prevention, Assessment and Tailored Health Screening (PATHS) Clinic. Pts with a personal history of BrCa prior to 1/2007 are excluded. Patients with a known genetic predisposition to BrCa, family history of breast cancer, or a personal history of LCIS, atypical hyperplasia or chest wall radiation are included in this analysis. All charts will be evaluated for documentation of the individual’s lifetime risk of BrCa and method used for risk-assessment, recommended and performed screening tests, concordance with ACS screening guidelines, patient adherence to initial and subsequent screening recommendations, and uptake of risk reduction strategies. Call-back rates for additional or follow-up imaging and/or biopsy following BrCa screening and characteristics of all new BrCa diagnoses will be collected.
Results: 240 eligible pts were seen during the study period. 15% of pts referred had a known genetic predisposition to BrCa. Most pts (75%) were referred for a family history of BrCa. The majority of these pts had a predicted lifetime risk of BrCa in excess of 20%, with less than 10% of patients being referred having a lifetime risk <20%. The remaining pts were referred for a personal history of LCIS, atypical hyperplasia or previous radiation to the chest wall. Results including subspecialty provider BrCa risk assessment, screening and risk reduction recommendations, patient uptake and adherence, outcomes of screening and characteristics of diagnosed BrCa cases will be presented.
Conclusion: Pts with a predicted increased lifetime risk for BrCa are often evaluated by oncology subspecialty providers. The primary factor related to referral is family history of BrCa. The majority of patients referred to a subspecialty provider have a calculated lifetime risk for BrCa in excess of 20%. This study evaluates provider assessment of BrCa risk and subsequent recommendations for screening and discussion of risk reduction strategies.
P4-11-22
Familial History of Cancer among Breast Cancer Women under 36 yr. in Rio de Janeiro, Brazil.
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Background. Antecedents of familial history of cancer (FHC) have been reported as an important feature observed among young women with breast cancer (BC). Objective. To explore the distribution of FHC in a sample of young women with BC in Brazil. Methods. Antecedents of FHC were obtained by a face to face interview carried out with 224 BC cases 20-35 yr., and 246 age-frequency matched controls, enrolled in a hospital-based case control study carried out among 1996-2006 in Rio de Janeiro, Brazil. Unconditional logistic regression was performed and BC odds ratios adjusted for age and education (adj. OR) were obtained according to FHC characteristics (parenthood degree, age of diagnosis and amount of relatives with cancer). Results. BC antecedents among 1st degree relatives was 5.4% among BC cases and 1.2% of controls (p=0.01). The adj. OR for BC among 1st degree relatives was 4.3, 95% CI 1.2-15.8. Comparatively to families without BC antecedents, an adj. OR =2.4, 95% CI 1.4-4.1 was observed for those with 1 reported BC case, and an adj. OR = 3.7, 95% CI 1.0-14.0 for those with 2 or more BC cases (p trend =0.0002). A similar trend was verified according to all other cancer sites (p-trend < 0.0001). The reported median age of BC diagnosis in the 1st generation (proband grand-mothers) was 76 yr., being 45 yr. at the 2nd generation (proband mothers) , and 38,4 yr. at the 3rd (proband sisters). A similar trend along generations was also verified for other cancer sites among case relatives, respectively, 69 yr., 58 yr. , and 45 yr. The mean age of diagnosis among the cases offspring was 6.7 yr. (standard deviation, SD = 0.6) and 12.0 yr. (SD = 1.4) among the controls descents (p< 0.01). Brain cancer reported among case relatives was 6.0 fold higher than among control relatives (3.7 fold higher for ovarian cancer, and 3.2 higher for skin cancer). Discussion. The observed results reveal that cancer risks among relatives of young women with BC are either higher for BC or other selected cancer sites. The observed decline along successive generations on the age at diagnosis of BC and other tumors is suggestive of recent genetic-environmental interaction in the studied sample of young women with BC in Brazil.

P4-12-01
The Breast-Activity and Healthy Eating after Diagnosis (B-AHEAD) Study – A Randomised Comparison of Weight Control Programmes during Adjuvant Treatment.
Harvie M, Pegington M, Bunded N, Campbell A, Wolstenholme J, Adams J, Speed S, Morris J, Howell A. University Hospital of South Manchester, Manchester; United Kingdom; University of Dundee, United Kingdom; University of Oxford, United Kingdom; University of Manchester; United Kingdom; University Hospital of South Manchester; United Kingdom

Background: Excess weight at diagnosis and weight gain during treatment are linked to increased mortality from breast cancer. Since 60% of breast cancer patients are overweight at diagnosis and 75% gain weight during treatment, weight control should improve outcome but the optimal intervention is unknown.

Aim: To compare 3 programmes for weight control after surgery for primary breast cancer.

Methods: We have recruited a randomised trial of 2 diet and exercise weight control interventions 1. a community based supervised group programme, 2. a mail and phone programme compared to standard written advice (control). We are comparing the relative effects of the 3 programmes on body weight and composition (by dual energy x-ray absorptiometry), cardiovascular risk parameters (lipids, blood pressure), a marker of breast cancer prognosis (insulin resistance), fitness and quality of life over the first year after diagnosis.

In total 409 women have been randomised (45% of eligible women) and 21 women have left the trial (5.1%). 357 have completed the 6 month assessments, all are due to complete the trial in December 2011.

Results: Weight and body fat results at 6 months indicate that the low cost mail and phone programme is equal to the supervised programme and both are significantly superior to written advice, but not in patients receiving chemotherapy.

The high uptake and adherence to the trial shows interest and motivation of a significant number of cancer patients to make positive changes to lifestyle at the time of diagnosis.

The final trial results will be presented at the December meeting.

This abstract presents independent research commissioned by the National Institute for Health Research under the Research for Patient Benefit programme. The views expressed here are those of the authors and not necessarily those of the funding organisations.

P4-12-02
Sereika SM, Dunbar-Jacob JM, Ryan CM, Adam B, Bender CM. University of Pittsburgh, Pittsburgh, PA; University of Pittsburgh Medical Center, Pittsburgh, PA

Background: Women with breast cancer experience deterioration in cognitive function with adjuvant therapy. Little is known about whether declines in cognitive function are associated with nonadherence to oral hormonal therapy over time. We investigated anastrozole adherence over time and its association with cognitive function over the first 12 months of therapy in women with breast cancer.

Methods: Using a prospective cohort design, 169 women with early stage breast cancer were monitored for 12 months 1) continuously for their adherence to anastrozole using electronic event monitoring and 2) objectively for cognitive function via a neuropsychological battery every 6 months for the first 12 months of hormonal therapy. Adherence data were summarized monthly as the percentage of prescribed doses taken and the timing of doses taken as the percentage of days with correct intake and the percentage of doses with correct timing. Six cognitive factors, derived via exploratory factor analysis applied to the neuropsychological battery, were used for analysis. Data were analyzed using descriptive and longitudinal methods.

Results: Participants were white (n=166, 98.2%), well-educated population. The views expressed here are those of the authors and not necessarily those of the funding organisations.
(Mean=15.1 years; SD=2.9, range=10-26) and on average 61.1 years of age (SD=5.9, range=46-75). Most had stage 1 breast cancer (n=123, 72.8%) and were prescribed anastrozole either alone (n=110, 65.1%) or immediately after chemotherapy (n=59, 34.9%). Initial levels of adherence were fairly high based on number of (Mean=89.1, SD=27.1, range=0-111.1) and timing of doses taken (Days with correct intake: Mean=86.4, SD=26.8, range=0-100; Doses with correct timing: Mean=83.6, SD=29.8, range=0-100); however, adherence decreased over the first 12 months of therapy (p<0.05) in terms of the number of and timing of doses taken and regardless of chemotherapy use. Specifically, we found a linear decrease for the percentage of prescribed doses taken (linear: t=-2.97, p=0.0035); however, nonlinear declines were observed for both the percentage of days with correct intake (linear: t=-3.33, p=0.0011; quadratic: t=2.10, p=0.0371) and the percentage of doses taken at the correct time (linear: t=-3.38, p=0.0009; quadratic: t=2.28, p=0.0242), suggesting a slowing of the rate of decline in adherence related timing of doses for months 9 through 12. We found that a decrease in executive functioning was related to lower percentages of prescribed doses taken (p=0.06) and doses taken at the correct time (p=0.06) from pretreatment to 6 months post-anastrozole initiation and even greater evidence (p<0.05) from pretreatment to 12 months post-anastrozole initiation. From 7 to 12 months post-anastrozole initiation, we found that a decrease in visual learning and memory was related to lower percentages of prescribed doses taken (p=0.04), days with correct intake (p=0.04), and doses taken at the correct time (p=0.03), while a decrease in attention was related to a lower percentage of days with correct intake (p=0.04).

Conclusions: These results suggest that breast cancer survivors who experience deterioration in cognitive function tend to have lower adherence. Efforts should be made to develop interventions to compensate for declines in cognitive function and to improve anastrozole adherence.

P4-12-03
Post-Diagnosis Weight Gain in Breast Cancer Survivors: When Should We Intervene?
Bradshaw PT, Cleveland RJ, Stevens J, Rosamond W, Abrahamson PH, Feitlbaum SL, Neugut AI, Gammon MD. University of North Carolina at Chapel Hill, Chapel Hill, NC; Mount Sinai School of Medicine, New York, NY; Columbia University, New York, NY

Significance. Weight gain after breast cancer diagnosis is common and has been linked to poor prognosis. Studies of the etiology and longitudinal pattern of post-diagnosis weight gain are limited, yet are critical to developing effective prevention strategies to enhance survival. Approach. We investigated the longitudinal pattern and determinants of post-diagnosis weight gain among 1,436 breast cancer survivors. The population-based cohort included women newly diagnosed with a first primary in situ or invasive breast cancer. Participants were randomized 1:1 to the WLI or educational control (EC) arm, with mean weight loss of 6.1% of body weight vs. 0.6% to loss of funding. The WLI arm lost significantly more weight than EC arm, with mean weight loss of 6.1% of body weight vs. 0.6% were more likely to gain weight during the first year after diagnosis [difference in mean yearly increase: BMI 25.0-29.9 vs. 18.5-24.9 (95% confidence interval): 1.93 kg/year (0.50, 3.37); BMI >=30.0 vs. 18.5-24.9: 0.47 kg/year (0.24, 0.71)] and after the first year [5.17 kg/year (3.68, 6.66) and 0.93 kg/year (0.58, 1.28), respectively], with the effect greater during the first year (p-interaction: <0.001). A pre-diagnosis weight gain of more than 10% since age 20 was also associated with post-diagnosis weight gain during year 1, difference in mean yearly increase compared to maintenance within 3% age 20 weight: 2.32 kg/year (0.59, 4.05); after year 1: 0.53 kg/ year (0.17, 0.89)] with the effect again stronger during the first year (p-interaction: 0.02). Modest associations, which varied only slightly with time, included: increases in post-diagnosis weight gain with chemotheraphy, tumor characteristics indicative of poor prognosis, and a previous diagnosis of hypertension, blood clots, or diabetes; and decreases with increasing recreational physical activity and a history of myocardial infarction.

Conclusions. Greater pre-diagnosis BMI and pre-diagnosis adult weight gain are strongly related to post-diagnosis weight gain among breast cancer survivors. The rate of post-diagnosis weight gain appears to be faster during the first year than after, suggesting that interventions to prevent post-diagnosis weight gain may be most important during the first year after diagnosis, especially among women who with BMI >= 25.0 1 year prior to diagnosis.

P4-12-04
Withdrawn by Author

P4-12-05
Impact of the Lifestyle Intervention Study in Adjuvant Treatment of Early Breast Cancer (LISA) Weight Loss Intervention upon Physical Activity.
Ligibel JA, Segal R, Pond G, Dion M-J, Pritchard KI, Levine M, Goodwin PJ. Dana-Farber Cancer Institute, Boston, MA; University of Ottawa, Ottawa, ON; McMaster University, Hamilton, ON; University of Toronto, Toronto, ON

Observational evidence shows a relationship between obesity and poor prognosis in breast cancer (BC). Physical activity (PA) is an important component of weight loss and maintenance, but most large-scale interventions in BC patients have produced only modest improvements in activity. We sought to evaluate changes in PA in women participating in LISA, a randomized trial coordinated by the Ontario Clinical Oncology Group which was designed to examine the impact of a telephone-based weight loss intervention (WLI) upon disease free survival in BC patients.

Methods:
Participants were randomized 1:1 to the WLI or educational control (EC) group. Eligibility included diagnosis of Stage I-III BC, BMI ≥24 kg/m², and treatment with letrozole. The WLI, based on the Diabetes Prevention Program, focused on weight reduction through calorie restriction and increased physical activity. Delivery involved 19 calls, mailings and a participant manual. The PA goal was 150 minutes/week. PA was measured using the International Physical Activity Questionnaire (Short Form) at baseline, 6, 12, and 18 months. Changes in time (minutes/week) spent sitting and engaging in moderate, vigorous, and walking activities were compared between groups.

Results
338 women were randomized to WLI (n=171) or EC (n=167) from 20 centers in Canada and the USA. The study was discontinued due to loss of funding. The WLI arm lost significantly more weight than EC arm, with mean weight loss of 6.1% of body weight vs. 0.6%
at 12 months (p<0.001). Activity data are presented in Table 1. At baseline, participants were inactive; median vigorous activity was zero minutes/week and median time spent sitting was more than 35 hours/week. WLI participants reported significantly higher participation in vigorous, moderate and walking activities, and lower levels of sedentary behavior, compared to controls at 6 and 12 months. Higher levels of PA were significantly associated with increased weight loss at all time periods. Factors associated with increases in activity included higher baseline BMI (p=0.014), lower baseline activity (p<0.001) and assignment to the WLI arm (p=0.02). Women assigned to WLI increased their PA during the intervention period (p=0.017) even after adjusting for other significant baseline factors.

Conclusion
Participants in the LISA WLI reported significantly higher levels of PA compared with controls. Activity increased most in women who were heavier and less active at baseline. Further study of this WLI as a potential means to improve BC outcomes is warranted.

P4-12-06
Risk Factors for Relative Weight Gain >10% in Breast Cancer Survivors: Findings from the SU.VI.MAX Cohort.
Zelek L, Czernichow S, Galan P, Hercberg S. Assistance Publique Hôpitaux de Paris, CHU Avicenne, Bobigny, France; INSERM U1125 INRA/CNAM/Université Paris 13, Bobigny, France
Purpose: Since extreme weight gain (EWG, defined as weight gain >10% of baseline body weight) raises risk for recurrence among breast cancer survivors (Caan et al. ACR 2011) we undertook this study to identify risk factors for EWG after breast cancer. Methods: We screened the database of the SU.VI.MAX trial (13017 pts randomized between low-dose antioxidants and placebo, Arch Int Med 2004) to retrieve women with confirmed breast cancer occurring after inclusion, and post-cancer follow-up. The following data were collected at inclusion and during the study: anthropometric measurements, physical activity, tobacco and alcohol consumption, level of instruction and socio-economic status, current medications. Unlike other breast cancer survivor studies, data have been collected prospectively before breast cancer diagnosis. Data management and data analysis were performed using SAS software 9.1.3. We also screened questionnaires and medical reports to collect data that were not recorded in the study database: type of adjuvant therapy (chemo- and hormone therapy), use of vitamins and dietary supplements, concomitant medications (such as antidepressants, lipid lowering agents, thyroid hormones or anti-thyroid drugs), previous use of HRT, and whether patients were following a diet plan after breast cancer or not.

Results: We identified 176 pts with breast cancer included since 1994 in the SU.VI.MAX 8-year Cohort Study. Among them, 25% (n=44) had weight gain >10% of baseline. The only variable significantly correlated with EWG was age at breast cancer diagnosis. Other differences, in particular level of physical activity or anthropometric measurements, did not reach statistical significance.

<table>
<thead>
<tr>
<th>Weight gain</th>
<th>≥-10%</th>
<th>≤-10%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at diagnosis</td>
<td>55.8±12.2</td>
<td>55.4±12.6</td>
</tr>
<tr>
<td>Age at inclusion</td>
<td>47.2±5.7</td>
<td>49.4±6.2</td>
</tr>
<tr>
<td>Non smoker</td>
<td>59%</td>
<td>55.6%</td>
</tr>
<tr>
<td>Alcohol drinker</td>
<td>88%</td>
<td>59%</td>
</tr>
<tr>
<td>Physical activity</td>
<td>33.8%</td>
<td>21.9%</td>
</tr>
<tr>
<td>walk≤1h/d</td>
<td>52.8%</td>
<td>36.1%</td>
</tr>
<tr>
<td>walk&gt;1h/d</td>
<td>31.8%</td>
<td>39.5%</td>
</tr>
</tbody>
</table>

No significant differences were seen between patients with EWG and others regarding the use of adjuvant chemotherapy (34 v 28%), aromatase inhibitors (18 v 22%), or tamoxifen (25 v 21%), previous HRT use (44 v 50%) or concomitant drugs including thyroid hormones (20 v 17%). A minority of patients was following a diet (16 v 17%) but consumption of vitamins or dietary supplements was slightly higher in patients without EWG (35 v 50%).

Discussion: Weight gain after breast cancer is a well-known phenomenon affecting at various levels half of breast cancer survivors. Most recent studies suggest that patients with EWG could have an increased recurrence rate. Risk factors for weight gain are still matter of debate but the role of adjuvant chemotherapy is widely admitted. Our results however indicate that adjuvant therapy does not seem to be a major risk factor for EWG. Furthermore, lifestyle and diet do not seem to markedly affect the risk of EWG. The only significant difference observed in our study is age at diagnosis, suggesting that treatment-induced amenorrhea could play a role in some patients. Although identification of women at risk remains difficult, thus precluding a large proportion of this population from tertiary prevention, younger women seem to deserve particular attention.

P4-12-07
Outcomes of a Behavioral Weight Control Intervention among Rural Breast Cancer Survivors.
Befort CA, Klemp JR, Austin HL, Krigel S, Sullivan DK, Schmitz KH, Perri MG, Fabian CJ. University of Kansas Medical Center; University of Pennsylvania; University of Florida
Background: Obese breast cancer survivors have 1.5 to 2.5 fold increased risk of recurrence and death compared to their normal weight counterparts. Rural women, who comprise over 20% of the U.S. population of women, have significantly higher obesity rates as well as breast cancer treatment-related disparities. Thus, weight control intervention is a key strategy for secondary breast cancer prevention in this population. However, access can be challenging in the rural setting. Using conference call technology to deliver group-based intervention is well-suited for rural breast cancer survivors because it is easily accessible and provides real-time peer support. The purpose of this one-arm treatment study was to examine the impact of a 6-month group phone-based behavioral weight control intervention on anthropomorphic, diet, physical activity, and psychosocial quality of life outcomes.

Methods: Eligible participants were post-menopausal breast cancer survivors (Stage I-IIIC, 3 months to 10 years since surgery, radiation, or chemotherapy, < 75 years of age, BMI 27-45 kg/m²) who resided in a rural area. The weight control intervention included a reduced calorie diet with 2 prepackaged meals and ≥ 5 fruit and vegetables servings daily, home-based physical activity gradually increased to 225 min/week of moderate intensity exercise, weekly self-monitoring logs, and weekly 60-minute group phone sessions that addressed...
behavorial modification and breast cancer survivorship topics. Group size ranged from 9 to 13 women. Measures included anthropometrics, two 24-hour dietary recall interviews, and questionnaires measuring physical activity, fatigue, depression, body image and sexuality, and self-efficacy for diet and physical activity behavior change.

Results: Participants (n = 34) were 58.9 ± 7.8 years-old, 3.1 ± 1.6 years out from treatment, had a baseline BMI of 33.7 ± 4.4 kg/m², and 63% were on anti-hormone therapy. Average sessions attendance among all participants, including 3 non-completers, was 90%. Ninety-one percent of participants (n = 31) attended > 75% of intervention sessions and completed post-treatment data collection visits. At 6 months, significant changes were observed for weight (−12.5 ± 5.8 kg, 13.9% of baseline weight), waist circumference (−9.4 ± 6.3 cm), daily energy intake (−349 ± 550 kcal/day), fruits and vegetables (+3.7 ± 4.3 servings/day), percent kcal from fat (−12.6 ± 8.6%), and physical activity (+1235 ± 832 kcal/week; all p's < .001). Significant improvements were also seen for Body Image subscales (Strength and Health, Social Barriers, Appearance and Sexuality), Depression, and Self-Efficacy for diet and physical activity behaviors (all p's < .05).

Discussion: The intervention produced significant improvements in weight, diet, physical activity, and quality of life outcomes that compare favorably to the literature. The group phone-based treatment delivery approach appears feasible and effective for weight control intervention among obese rural breast cancer survivors.

P4-12-08
Five Year Preliminary Outcomes of a Prospective Surveillance Model To Reduce Upper Extremity Morbidity Related to Breast Cancer Treatment.

Stout NL, Pfalzer L, Levy E, McGarvey C, Gerber L, Springer B, Soballe P. National Naval Medical Center; University of Michigan-Flint; National Institutes of Health; CLM Consulting; George Mason University; Office of the Surgeon General; Naval Hospital San Diego

Background: Early detection and management of physical impairments after breast cancer treatment contribute to successful functional outcomes and improved quality of life throughout disease treatment and survivorship. Assessment of upper extremity (UE) morbidity including; shoulder dysfunction, scarring, pain, fatigue and lymphedema should be conducted through a prospective surveillance model of care to promote early identification of impairments and provide intervention while functional limitations are minimal, thereby preventing long term loss of function. This report highlights 5-year findings related to physical function in patients participating in a prospective surveillance model of care.

Methods: A prospective, observational study enrolled women with breast cancer at the point of disease diagnosis (n=196) and measured UE morbidity, impairments and functional disability over a 5 year period. Patient demographics, cancer characteristics, measures of UE strength, range of motion (ROM) and limb volume were taken pre-operatively and repeated at 1, 3, 6, 9, 12 and 60 months post-operatively. Subjective assessment of physical activity, health status and quality of life were assessed by questionnaire at 12 and 60 months. 166 subjects completed visits at 1 year and 95 completed visits at 5 years. All subjects received education regarding exercise, risk reduction and advice on return to activity. If physical impairments were detected during the study, immediate physical therapy intervention was initiated to alleviate the impairment.

Results: The incidence of objective UE impairments at five years after treatment was 9% with loss of shoulder ROM, 25% with subclinical lymphedema (defined as ≥ 3% change in limb volume from baseline), 5.6% with advanced lymphedema (Stage I or II) and 27.8% with clinically significant fatigue (defined as ≥ 3 on a visual analog scale). Subjectively 8.4% reported feeling moderately or severely disabled with their affected arm, 11.1% reported moderate to severe difficulty carrying heavy objects, 4.2% reported moderate to severe limitations with heavy household chores.

Discussion: This is the first prospective cohort study in the United States to specifically monitor physical and functional outcomes to 5-years post breast cancer treatment. The prospective surveillance model of care, conducted by the physical therapist, enabled early detection and treatment of breast cancer treatment–related impairments resulting in improved long-term function. Long-term incidence of UE morbidity after breast cancer treatment has been documented in the literature as high as 40-60% with lymphedema and up to 60% with fatigue. This study clearly demonstrates the potential for substantial reduction in UE dysfunction related to breast cancer treatment when using an early identification and intervention model. Morbidity such as pain, reduced range of motion, decreased strength and sub-clinical lymphedema were detected early and managed through the prospective model. These results strongly suggest that prospective surveillance monitoring for functional impairments is an optimal construct to assure long-term function in women after breast cancer treatment.

P4-12-09
Withdrawn by Author

P4-12-10
A Prospective Study of Physical Activity Patterns and Changes in Breast Cancer Patients during Active Breast Cancer Treatment.

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Purpose

Physical activity (PA) has been shown to benefit cancer patients’ physical functioning, emotional well-being, and symptom management. Moderate PA may reduce the side effects of breast cancer treatment and improve quality of life, but most of the studies have been conducted after active treatment was completed and there is little data on patterns of PA during active treatment. This study aims to evaluate patterns and changes in physical activity among breast cancer patients during active treatment.

Patients and Methods

We recruited 411 women with non-metastatic breast cancer between July 2010 and Dec 2010 from two major cancer hospitals in Seoul, Korea. Trained researchers interviewed participants at enrollment (before surgery), and at 2 weeks, 3 months, and 6 months post surgery. Intensity and duration of PA were assessed using the Minnesota Leisure Time Physical Activity Questionnaire, and energy expenditure (the average calories spent per day) was calculated as metabolic equivalents (METs). Quality of life (EORTC), fatigue (BFI), socio-demographic and clinical characteristics were also assessed. After excluding 15 patients with recurrence during follow-up and 65 patients were lost to follow-up, the final sample size was 331 patients (80.5%).
Results
The mean (SD) age and BMI of study participants were 46.4 years and 23.3kg/m² (SD 3.35). 87.3% of women had lumpectomy, and 70.6% and 85.9% had adjuvant chemotherapy and radiotherapy respectively. Breast cancer patients were most likely to spend their energy in lifestyle (walking and driving) and leisure (shopping, reading, and watching TV) activities but spent little energy in sport activities (Table).

<table>
<thead>
<tr>
<th>Energy Expenditure (Kcal/Day)</th>
<th>Baseline</th>
<th>2 weeks Post-surgery</th>
<th>3 months Post-surgery</th>
<th>6 months Post-surgery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lifestyle activities</td>
<td>250.8±32.9</td>
<td>314.5±39.1</td>
<td>340±42.6</td>
<td>438±54.4</td>
</tr>
<tr>
<td>Leisure</td>
<td>624.2±13.2</td>
<td>627±17.4</td>
<td>606±19.0</td>
<td>594.2±28.3</td>
</tr>
<tr>
<td>Household chores</td>
<td>191.3±26</td>
<td>161±18.6</td>
<td>249±20.4</td>
<td>235.6±26.3</td>
</tr>
<tr>
<td>Sport</td>
<td>82.5±12.7</td>
<td>80.9±13.9</td>
<td>82.8±15.0</td>
<td>113.9±19.1</td>
</tr>
<tr>
<td>Total</td>
<td>1674.4±43.7</td>
<td>1610.3±49.6</td>
<td>1331.6±54.0</td>
<td>1398.3±70.3</td>
</tr>
</tbody>
</table>

Conclusion
PA declined after the surgery and gradually recovered at 3 and 6 months post surgery but it did not return to the baseline level. Breast cancer patients may need encouragement and support to perform more intensive physical activities during active treatment.

*Acknowledgement: The research was accomplished by the support from AMOREPACIFIC and Korea Breast Cancer Foundation.

P4-12-11
Myelodysplastic Syndrome Post Primary Breast Cancer Treatment: Cases from a Community Cancer Center: 1990-2010.
Kaplan HG, Malmgren JA, Atwood MK. Swedish Cancer Institute, Seattle, WA; HealthStat Consulting, Inc., Seattle, WA

Background: Myelodysplastic syndrome (MDS) has not been well documented or described post breast cancer (BC) treatment.

Methods: A cohort of all breast cancer patients from 1989 to 2010 at our institution were followed for incidence of blood disorders of any type by our registry system and by linkage to the Surveillance, Epidemiology and End Results (SEER) registry for our area. All cases of post breast cancer leukemia were reviewed for incidence of MDS from a cohort of 9846 cases diagnosed between 1990 and 2010. Coding of cases was assigned by the SEER registry using ICD-0 codes for type of leukemia from pathology reports. 20 cases of MDS were identified and confirmed using SEER coding for MDS and death certificate review if applicable. Variables of interest included age, race, treatment received, recurrence and treatment for recurrence, time interval from breast cancer diagnosis to leukemia diagnosis and time from leukemia diagnosis to follow up or death. Cytogenetic studies were available on 5 of the 20 patients.

Results: Mean age of the patients was 56 years, range 33-79, with 65% < age 65. 90% of the patients were white and 10% Asian. TNM BC stage distribution was stage I=0, stage II=8, and stage III=2. MDS was diagnosed at a median time post BC diagnosis of 65 months, range 8-205 months including 5 patients treated for recurrence and median 47 months, range 8-205 months excluding patients with recurrence. Nine patients had initial chemotherapy treatment, two without initial adjuvant therapy received chemotherapy for recurrence, and 9 received radiation only. Eight of eleven patients who received chemotherapy were treated with doxorubicin. All eleven received cyclophosphamide. Five of the 20 cases had MDS, not otherwise specified (NOS) (9989), 4 cases had MDSaAML, 3 MDS treatment related (9987), 3 refractory anemia (RA) (9980), 3 refractory anemia with excess blasts (RAEB) (9983), 1 RA with sideroblasts (9982), and 1 MDS with 5q deletion. Three of the five patients with cytogenetics available had chromosome 7 abnormalities and one had 11q21.23.

Eleven of the 20 patients (55%) died of MDS or MDSaAML with a mean survival of 17 months, range 0.5-72 months. Six patients were alive at follow up with mean survival 56 months, range 43-89. Two of the surviving patients had stem cell transplants. The remaining three patients died of other causes with mean survival 66 months, range 7-109 months. Mean survival of all patients was 36.26 months, range 53-109 months with a significant difference in survival time between the patients that died of MDS, those alive at follow up and those that died of other causes (F statistic 6.56, p=.008).

Discussion: MDS is a significant complication of breast cancer treatment with radiation and/or chemotherapy with a high rate of associated mortality.

P4-12-12
Patient-, Illness-, and/or Treatment-Related Baseline Predictors of Nonadherence to Oral Hormonal Therapy.
Wickersham KE, Sereika SM, Bender CM. University of Pittsburgh, Pittsburgh, PA

Background: Nonadherence to oral hormonal therapy is problematic for women with breast cancer. Patient-, illness-, and treatment-related factors have been associated with nonadherence, but with inconsistent findings. Therefore, our aim was to explore predictors of nonadherence to hormonal therapy for women with early stage breast cancer from the baseline assessment (pre-hormonal therapy) to 6 months post-treatment.

Methods: A secondary analysis was performed to explore potential patient-, illness-, and treatment-related predictors of nonadherence for 198 women enrolled in either: 1) The Anastrozole Use in Menopausal Women Study (AIM Study, n=162), or, 2) Predictors of Adherence to Hormonal Therapy in Breast Cancer (ONS Study, n=36). Nonadherence was defined as the percentage of prescribed administrations of hormonal therapy that were not taken during the first 6 months of therapy as measured using an electronic Drug Exposure Monitor (eDEM) (AARDEX, Ltd.). Chi-square tests of independence and Mann-Whitney U tests were performed to determine whether data from the two studies could be pooled. Descriptive statistics were performed to characterize the sample. Multiple linear regression analyses were performed to identify the best model in two stages: 1) univariate relationships between each candidate predictor variable and the outcome variable (6-month nonadherence) were assessed using a cut-off of p=.20; and, 2) candidate predictors meeting the criteria were retained for further exploration in model-building multiple linear regression analyses (stepwise and backward) to determine the predictors of 6-month nonadherence summary scores. Candidate predictors were retained in the model if they remained associated at p<.10 in the multiple regression analysis.

Results: Women were 98.3% Caucasian with a mean age 59.1 years (SD 7.5) and mean number of years of education of 15.0 (SD 2.9). Overall mean nonadherence was 11.6% (13.2% AIM Study, 4.6% ONS Study). Chi-square and Mann-Whitney U tests demonstrated that the two samples could be pooled, given the data were similar on key variables (number of years of education, depression, anxiety, fatigue, symptoms, and nonadherence). Both stepwise and backward elimination modeling algorithms demonstrated evidence of 3 significantly significant variables associated with nonadherence; however, the backward elimination model best represented the sample (R²= .106, adjusted R²= .086, s=.28490). Women who worked (p=.082), whose primary occupation was clerical or administrative (p=.029), had DCIS tumor type (p=.017), or who had higher gastrointestinal (GI) symptoms scores (p=.013) were associated with nonadherence. The potential for interactions between primary occupation, DCIS tumor type, and higher GI symptoms was explored and was not significant. In addition, while all tumor types were...
examined as candidate predictors, participants with DCIS also had another tumor to be eligible for the parent studies.

Conclusions: Our study offers insight into potential predictors of nonadherence for women participating in one of two cohort studies. The findings suggest additional examinations of nonadherence concerning work and symptom burden and their relationship to nonadherence are indicated.

P4-12-13
A Multi-Center Randomized Controlled Double Blind Trial Assessing the Effect of Acupuncture in Reducing Musculoskeletal Symptoms in Breast Cancer Patients Taking Aromatase Inhibitors: First Interim Analysis.


Background: Aromatase inhibitors (AIs) are recommended as first-line adjuvant hormonal therapy in postmenopausal women with hormone-receptor–positive breast cancer, as monotherapy or sequential therapy after tamoxifen. AI-associated musculoskeletal symptoms (AIMSS) occur in approximately 50% of women receiving AIs and in some may result in discontinuation of treatment. Symptom management is essential to ensure that breast cancer patients receive the full recommended duration of AI therapy. We conducted a randomized, placebo-controlled trial to evaluate the effect of acupuncture on AIMSS and report the first interim analysis.

Method: Postmenopausal women with early stage breast cancer, experiencing AIMSS, who had not had acupuncture in the year prior to the study, were eligible. Patients were randomized to 8 weekly acupuncture or sham acupuncture. Health assessment questionnaire disability index (HAQ-DI) ranging 0-3.0) and pain visual analog scale (VAS ranging 0-100) were used to assess clinical musculoskeletal disorder severity at weeks 0, 4, 8, and 12 or 24. Change in HAQ-DI (ΔHAQ-DI) and VAS scores (ΔVAS) from baseline were compared between patients receiving acupuncture versus sham acupuncture using exact Wilcoxon rank sum test. Serum samples were collected for measurements of estrogens and beta endorphin concentrations and cytokine profile before and after the intervention to evaluate the etiology of AIMSS and the mechanism of acupuncture in treating AIMSS.

Results: Between May 2008 and June 2011, 48 patients were enrolled, 2 patients were not evaluable due to noncompliance to treatment and lost to follow up, 10 were still receiving treatment and therefore not evaluable. Thirty-six were evaluable, and were equally distributed between the real and sham acupuncture groups. Baseline characteristics were balanced between the two groups with regard to age, race, and body mass index (BMI) with the exception that baseline mean HAQ-DI was higher in the acupuncture group (0.9 vs 0.55, p = 0.04). White/Black/Asian: 26/7/3. Median (range): age: 61 (45-82); BMI (kg/m²): 31 (22.9-59.6). At week 8, both groups showed a wide range of ΔHAQ-DI (ΔHAQ-DI = HAQ-DI week8 - HAQ-DI baseline): from -1.38 to 0.5 in the acupuncture group versus from -1 to 0.12 in sham acupuncture group. There was no statistically significant difference in mean ΔHAQ-DI between the real and sham acupuncture groups (-0.33 vs -0.33, p=0.87). Eleven patients in each group (61%) reported decreased HAQ-DI scores, which correlated with improved function. There was no difference in mean ΔVAS between the real and sham acupuncture groups (-9.27 vs -13.82, p=0.67). No significant side effects were reported. Changes in other time points and in serum biomarkers will be presented at the meeting.

Conclusions: The majority of breast cancer patients experiencing AIMSS who participated in our study reported a reduced HAQ-DI score both from acupuncture and sham acupuncture. We did not observe significant differences between responses to real versus sham acupuncture after 8 weekly treatments. The study remains open to accrual to reach 50 evaluable patients.

P4-12-14
Pilot Study Utilizing Fluorine-18 Fluorodeoxyglucose (F-18 FDG) Positron Emission Tomography–Computed Tomography Scan (PET-CT Scan) To Investigate Brain Metabolic Changes during Treatment in Women with Breast Cancer.


BACKGROUND: Approximately 25% of the patients undergoing chemotherapy develop cognitive changes. Similarly, significant neurocognitive changes in verbal memory and executive functioning have been reported in patients undergoing endocrine therapy. Though extensively studied, specific changes in the brain associated with cognitive dysfunction are still not clear. We performed an IRB-approved retrospective pilot study utilizing brain images from standard PET-CT scans in patients being treated for breast cancer. Comparison was made between patients initial and follow-up scans to look for metabolic changes.

MATERIALS AND METHODS: Thirty nine patients with a diagnosis of breast cancer were identified from radiology database of West Virginia University Hospitals, who underwent at least two PET-CT scans during their treatment for breast cancer from 2004-2009. Patients with brain metastasis were excluded. NeuroMIM® software analysis program was used to compare a comprehensive database of physiologic brain anatomy and metabolism with F-18 FDG perfusion brain images from the patients. Comparison was made in sixty-three defined brain regions. For each patient, two scans at approximately twelve month intervals were analyzed. The data sets from initial scans were compared with the follow up.

RESULTS: A total of 37 patients received cytotoxic chemotherapy, 2 patients received only endocrine therapy. Data analysis using the signed-rank test shows that the collective Z-score values change between the initial and follow up scans. When data analysis is applied to the individual brain regions, the Lingual Gyrus (p=0.012) and the Angular Gyrus (p=0.056) show statistically significant and near significant decreases in brain metabolism respectively. These regions are attributed with language, mathematics and cognition. Several additional regions such as the fusiform gyrus and the primary visual cortex show p-values between 0.05 and 0.10, which indicate “trending”. These regions may demonstrate statistically significant decrease in metabolism if the sample size is increased.

DISCUSSION: The Lingual and the Angular Gyrus show a statistically significant and near significant decrease in glucose metabolism respectively, in patients receiving treatment for breast cancer. Limitations of this study include lack of baseline brain imaging and its clinical correlation with cognitive function. Based upon these preliminary findings prospective studies are being planned.
**P4-12-15**

**Cancer Survivor Care: An Evaluation of a Group Visit Model of Care for Breast Cancer Survivors at Duke Cancer Center.**

*Trotter KJ, Schneider S. Duke University Medical Center; Durham, NC; Duke University, Durham, NC*

**Background:** The number of cancer survivors is growing, currently with about 2.5 million breast cancer survivors. (ACS, 2010). The Institute of Medicine report on survivor care stressed care coordination along with attention to survivor concerns as key issues in improving follow-up care. Group visits have been shown to improve access, health outcomes and health care utilization rates, as well as self-management skills.

**Purpose:** This prospective program evaluation measured patients opinions about participation in a group visit clinic.

**Methods:** A new model of breast cancer survivor care was designed and piloted at Duke Cancer Center. Breast cancer survivors who were three years post diagnosis attended the clinic together in groups of six. An interdisciplinary group visit format in the initial part of the appointment provided surveillance, education and support, as well as formation of an individualized survivorship care plan to be shared with the primary care provider. The first hour included review of their personal care plan and a 45-minute facilitated discussion. Afterwards, individual visits with the nurse practitioner, and dietitian, physical therapist or social worker occurred.

A 22-item questionnaire designed to ascertain opinions regarding logistics and the style and function of care delivered is being administered to participants. The evaluation tool was approved by a panel of experts; pilot tested and has a reading level of 7.8. Data collection will be complete July 1, 2011. 103 surveys have been collected with a 91% response rate. Preliminary analysis shows high satisfaction with the group visit format. What participants liked most about the program was sharing with other survivors. 86% of participants indicate that they will share the survivorship care plan with their PCP. Only 10% responded that they were not sure they needed this type of follow up now and expressed that it would be more helpful immediately after finishing treatment. Final data will be presented. Barriers and facilitators to implementing this type of care model will be presented.

**Conclusions:** The group visit model applied to a breast cancer survivor clinic appears feasible and highly satisfactory to participants.

**Implications for practice:** Group visits can be considered one option for survivor care.

**P4-13-01**

**Pain Severity and Analgesic Use Associated with Skeletal-Related Events in Patients with Advanced Breast Cancer and Bone Metastases.**

*Fallowfield L, Cleeland CS, Body J-J, Stopeck A, von Moos R, Patrick DL, Clemons M, Tonkin K, Masuda N, Lipton A, De Boer R, Salvagni S, Tosello Oliveira C, Ying W, Braun A, Cong Z. Cancer Research UK, University of Sussex, Brighton, UK; University of Arizona, Arizona Cancer Center, Tucson, AZ; University Libre de Bruxelles, Brussels, Belgium; University of Texas, M.D. Anderson Cancer Center, Houston, TX; CHU Brugmann, Université Libre de Bruxelles, Brussels, Belgium; University of Arizona, Arizona Cancer Center; Tuscon, AZ; University of Washington, Seattle, WA; The Ottawa Hospital Cancer Centre, Ottawa, ON, Canada; Cross Cancer Institute, Edmonton, AB, Canada; Osaka National Hospital, Osaka, Japan; Penn State Milton S. Hershey Medical Center, Hershey, PA; Royal Melbourne Hospital, Melbourne, Australia; Azienda Ospedaliera di Parma, Parma, Italy; Instituto Brasileiro de Controle do Cancer-IBCC, Sao Paulo, Brazil; Amgen Inc., Thousand Oaks, CA*

**Background:** Skeletal-related events (SREs), which include pathologic fracture (PF), radiation to bone (RB), surgery to bone (SB), and spinal cord compression (SCC), occur frequently in patients with bone metastases and can lead to debilitating clinical consequences such as functional impairment and bone pain. Denosumab (XGEVA®) is a fully human monoclonal antibody against RANKL, shown to be superior to zoledronic acid (ZA; Zometa®) for the prevention of SREs in patients with solid tumors. Denosumab also delayed the onset of moderate or severe pain compared with ZA. The pain severity and analgesic use associated with each type of SRE were assessed in patients with advanced breast cancer and bone metastases.

**Methods:** Eligible patients received denosumab 120 mg SC or ZA 4 mg (adjusted for renal function) IV every 4 weeks in a randomized, multinational, double-blind, double-dummy trial. Patient-reported pain was assessed with the Brief Pain Inventory (BPI; 0 no pain to 10 severe pain) at baseline (BL) and at each monthly visit. Opioid and non-opioid analgesic use was recorded and scored using the Analgesic Quantification Algorithm (AQA; 0 no analgesic use to 7 > 600mg oral morphine equivalent/day). Data from the two treatment arms were pooled for this analysis. Pain and analgesic use were evaluated from 6 months prior to and 6 months after the first on-study SRE for each patient. The comparator group included patients without an SRE and was centered at the median time from randomization to first SRE by SRE type, with corresponding 12 month assessments. The proportion of patients with moderate/severe pain (BPI worst pain score > 4) and proportion of patients shifting from no/low analgesic use (AQA ≤ 2) at baseline to strong opioid use (AQA ≥ 3) were reported by month and by SRE type, and are summarized by mean relative change (%) across the time period.

**Results:** In total, 687 patients with first on-study SRE occurrences (PF=450, RB=201, SCC=16, SB=20) were analyzed. A similar proportion of patients with and without a PF had moderate/severe pain, but a higher proportion of patients with a PF shifted from no/low analgesic use to strong opioid use (mean relative increase 80%). Starting 3 months prior to the event, more patients with RB than those without had moderate/severe pain (27% mean relative increase) and shifted from no/low analgesic use to strong opioid use (mean relative increase 269%). Similar pain and analgesic use patterns were noted for patients with SCC (mean relative increase in pain: 63%; in AQA shift: 913%). More patients with SB than those without had moderate/severe pain in the 6 months leading on to SB (mean relative increase 51%). The difference was attenuated after SB, but during this time a much higher proportion of patients with SB shifted from no/mild opioid use to strong opioid use (mean relative increase 22%).
Discussion: SREs are associated with increased pain severity and analgesic use in patients with advanced breast cancer and bone metastasis. Patterns of pain severity and analgesic use differed by SRE type. Effective treatments to prevent SREs can decrease pain and the need for treatment with opioid analgesics.

**P4-13-02**

**Hospice Utilization and End of Life Care in Metastatic Breast Cancer Patients at a Comprehensive Cancer Center.**

O’Connor TL, Ngamphaiboon N, Morris J, Callinan NK, Milch RA, Kerr CH, Watroba N, Edge SB. Roswell Park Cancer Institute, Buffalo, NY; The Buffalo Center for Hospice and Palliative Care, Cheektowaga, NY

Background: Metastatic breast cancer patients have many options for therapy and may be at risk for late or absent hospice referrals. Previous studies suggest that late referrals to palliative care make meaningful improvements in symptoms and quality of life difficult to achieve. We examined hospice utilization, status of patients on hospice admission, and quality of care documentation in patients treated for metastatic breast cancer at Roswell Park Cancer Institute (RPCI) from 1999-2010.

Methods: Analysis of breast cancer cases through the RPCI database identified 182 cases with deaths resulting from breast cancer eligible for services through The Buffalo Center for Hospice and Palliative Care (HB) (residence in Erie County, NY). Cases were cross-matched to the HB database for information on hospice utilization and outcome, and patient symptoms/status on admission to hospice. Date of last chemotherapy, opiate use, anxiolytic use, documentation of advance directive and palliative care discussion, and place of death were collected through chart abstraction.

Results: 182 patients eligible for HB services expired from breast cancer during the study period. 122 (67%) utilized hospice services; 60 (33%) did not. 117 patients received chemotherapy as their last treatment for metastatic breast cancer; patients referred to hospice received their last dose of chemotherapy a median of 31 days (14.1-47.9) prior to death; non-hospice patients 16 days (0-44.1) p=0.071. Patients subsequently admitted to hospice were significantly more likely to have a discussion of advance directives by their oncology team (30% vs. 5% p<0.001) and were more likely to die at home (56% vs. 8%, p<0.001). Only 7% of the 60 patients who died without a hospice referral had a documented discussion of palliative care as a therapeutic option by the oncology team (p<0.001) versus 100% of patients referred to hospice. 79% of breast cancer patients not admitted to hospice died in an acute care setting. Prior to hospice admission or death, 38% (69) patients had documentation of long-acting opiate use; 72% (131) were prescribed a short acting opiate, while 29% (52) patients were prescribed an anxiolytic. Median length of stay (LOS) was 17 days; 14% of patients died within 3 days of admission. Patient status on hospice admission is reported in Table 1.

| Status on HB admission (n=122) |
|--------------------------|--------------------------|
| Age                      | 77 (27%) |
| Pain Score               | 0-1 (15%)
| 2-5 (30%)
| 6-10 (21%)
| missing                  | 14
| Palliative Performance Score (PPS) |
| 10-20                    | 15 (11%)
| 30-40                    | 63 (50%)
| 50-60                    | 32 (26%)
| missing                  | 19
| 8/10 dependent children  | 1 (0.8%)
| 0                        | 76 (62%)
| 1                        | 16 (13%)
| 2                        | 5 (5%)
| 3                        | 8 (6.6%)
| missing                  | 17
| Palliative care          | 79 (65%)
| documentation missing    | 43 (35%)
| Pain Score               | 0-1 (15%)
| 2-5 (30%)
| 6-10 (21%)
| missing                  | 14
| Palliative Performance Score (PPS) |
| 10-20                    | 15 (11%)
| 30-40                    | 63 (50%)
| 50-60                    | 32 (26%)
| missing                  | 19
| 8/10 dependent children  | 1 (0.8%)
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| 10-20                    | 15 (11%)
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| 1                        | 16 (13%)
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| 3                        | 8 (6.6%)
| missing                  | 17
| Palliative care          | 79 (65%)
| documentation missing    | 43 (35%)
| Pain Score               | 0-1 (15%)
| 2-5 (30%)
| 6-10 (21%)
| missing                  | 14

Conclusion: Despite the recognition of the importance of high-quality end of life care, including end of life decision-making, advance directives, pain and symptom management, and hospice care, 33% of metastatic breast cancer patients at our institution died without hospice referral. Those patients referred to HB were significantly more likely to have an advance directive discussion, and to die at home. However, patients referred to HB had a median LOS of 17 days, and poor PPS. Efforts to enhance discussion of palliative care earlier in the treatment of MBC are critical to improved patient care.

**P4-13-03**

**Retrospective Analysis of Palonosetron Compared with Older 5-HT3 Receptor Antagonists (Ondansetron, Dolasetron, and Granisetron) in Preventing Anthracycline-Induced Nausea and Vomiting in Breast Cancer Patients from Four Phase 3 Trials.**

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In patients with breast cancer, the risk of developing chemotherapy-induced nausea and vomiting (CINV) is particularly high due to the common use of highly emetogenic chemotherapy coupled with pt-related risk factors including female gender and young age. Despite prophylactic antiemetics, controlling CINV is difficult in this high risk population, particularly in the delayed phase following CT. Palonosetron, a potent 5-HT3 receptor antagonist (RA) with a distinctly different pharmacokinetic and receptor binding profile, has demonstrated improved CINV protection compared to older 5-HT3 RAs in phase 3 and 4 clinical trials in a variety of settings and tumor types.

We conducted a retrospective subset analysis of pts with breast cancer, the majority of whom (89%) received anthracycline combination chemotherapy (doxorubicin+epirubicin+cylophosphamide, AC/EC) from four phase 3, randomized, double-blind, parallel group studies to assess the comparative effectiveness and safety of single IV doses of palonosetron (0.25 or 0.75 mg) versus older 5HT3-RAs (ondansetron 32 mg, dolasetron 100 mg, and granisetron 3 mg) in preventing CINV in pts receiving this highly emetogenic chemotherapy. The primary efficacy evaluation was for complete response (CR), defined as the proportion of patients with no emetic episodes & no use of rescue medication during 0-24hrs (acute), >24-120hrs (delayed), & 0-120hrs (overall) using a logistic regression model. A comparative descriptive safety assessment was also performed.

A total of 1197 patients with breast cancer were included in this analysis (palonosetron n=714; older 5HT3-RAs n=483). Higher CR
rates were seen for palonosetron vs. older 5HT3-RAs during the acute (63.4% vs 59.4%, p= 0.08), delayed (59.2% vs. 44.9%, p=0.0001) and overall (50.7% vs.38.5%, p<0.0001) phases. Rates for CR were similarly higher for palonosetron-treated patients in the large subgroup of patients receiving AC/EC based chemotherapy. Consistent improvements associated with palonosetron were seen for secondary endpoints of complete control (CR + no more than mild nausea), no emesis, and severity of nausea. The adverse event (AE) profile for palonosetron was similar to older 5HT3-RAs, with headache and constipation being the most commonly reported treatment-related AEs with similar incidences for all 5-HT3 RAs. This retrospective analysis of patients with breast cancer, including a large subgroup receiving anthracycline-based highly emetogenic chemotherapy, demonstrated significantly improved CINV prevention with palonosetron compared to older 5HT3-RAs, particularly in the delayed phase. Safety was comparable. This data has important implications for current CINV prevention guidelines.

### P4-14-01

**Weight-Adjusted Change of Unilateral Arm Volumes for Quantification of Lymphedema after Bilateral Breast Surgery.**

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**Background:** Accurate quantification of breast cancer-related lymphedema (BCRL) is important for early detection and successful management. Arm volume changes after unilateral breast surgery can be calculated through relative volume change (RVC) of the affected arm using the contralateral arm as a control (Ancukiewicz et al Int J Radiat Oncol Biol Phys 79(5):1436-43 2011). However there currently exists no accurate method for quantifying arm volume changes in patients after bilateral breast surgery. As the number of women undergoing bilateral mastectomies continues to increase, it is critical to develop a method to quantify arm volume changes in this population for detection of lymphedema. Our aim was to develop a weight-adjusted formula for unilateral arm volume changes independent of the contralateral arm for application in the setting of bilateral breast surgery.

**Materials and Methods:** We analyzed longitudinal measurements of arm volumes in 141 unilaterally affected patients undergoing screening for BCRL with a perometer at our institution (71 with left affected and 70 with right affected arms). Each patient had a baseline measurement prior to surgery and at least one post-surgical measurement. Median time of follow-up was 27.5 months and median standard deviation of longitudinal weight changes within patients during follow-up was 3%.

**Results:** Unilateral arm volume changes of the unaffected arm were correlated with weight changes at the last follow-up point (Kendall’s tau=.58 and P<.0001). Estimates of slope and intercept for median regression line between percent weight change and percent arm volume change in the unaffected arm for application in the setting of bilateral breast surgery.

**Discussion:** Unilateral arm volume changes can be quantified by adjusting for weight changes. We propose the weight-adjusted change (WAC) formula to calculate unilateral arm volume changes for detection and monitoring of BCRL in patients who undergo bilateral breast surgery. The validity of such a formula needs to be assessed in conjunction with clinically reported lymphedema.
was a transient increase in LE. However, overall, we speculate that the higher risk for LE after 1 year is because of lack of use of the non-dominant affected arm. It is use of the upper limb which is likely the critical issue in this first year. Exercise was preventative while it was being undertaken; we question whether women maintained the level of exercise once the intervention concluded.

Akknowledgement: Cancer Council NSW; NBCF Australia

P4-14-03
Support Groups in Breast Cancer: An Evidence Based Assessment of 1606 Patients with Concerning Topics for Support Group Discussion and Presentation.
Gralla RJ, Morse KD, Rittenberg CN, Petersen JA, Rosen LM, Lesser M. Hofstra North Shore - LIJ School of Medicine, New York, NY; Nexcura, Seattle, WA; Feinstein Institute for Medical Research, Manhasset, NY

Background: Cancer support groups are conducted at most cancer centers. Although there is increasing demand among patients and families for support groups, little data exist outlining which topics patients and caregivers consider as most important for support group discussion and presentation. We conducted a large web-based anonymous survey to determine preferences of patients and caregivers for cancer support groups, including 1606 individuals with breast cancer and 3723 with other malignancies.

Methods: The established patient base of NexCura®, a web-based information resource, was used to survey registered patients and caregivers in this on-line assessment. Participants ranked 26 topics on a 5-point Likert scale which evaluated the importance of each item. Demographic characteristics, clinical factors, and support group content preferences were assessed.

Results: There were 4402 respondents to the invitation to participate, and 3723 patients and caregivers elected to participate (85%). Characteristics included for all 3723 patients: 70% women, mean age 58 (range 20-89). For the 1606 patients with breast cancer, 99.8% were women, the mean age was 56 (range 27-88); 53% were free of cancer. For the whole group, the four most common cancers were breast 45%, prostate 15%, lung 6%, and colorectal 5%. 90% of participants were women, the mean age was 58 (range 20-89). For the 1606 patients with breast cancer, 99.8% were women, the mean age was 56 (range 27-88); 53% were free of cancer.

Topics similarly to the general cancer population; however, topics dealing with fatigue and stress management were somewhat more highly ranked by those with breast cancer. 3) Physical, psychological, and communication issues were the most highly ranked issues by breast cancer patients and families. 4) These results should guide the content of support groups to meet the needs of patients and caregivers.

Table 1

<table>
<thead>
<tr>
<th>TOPICS (Top 10 rated of 26)</th>
<th>BREAST CANCER PATIENTS</th>
<th>ALL PATIENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=1606</td>
<td>n=3723</td>
<td></td>
</tr>
<tr>
<td>Cancer treatment choices</td>
<td>94.8% (1)</td>
<td>95.8% (1)</td>
</tr>
<tr>
<td>%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Side effects and their prevention</td>
<td>84.5% (2)</td>
<td>93.3% (2)</td>
</tr>
<tr>
<td>%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Living with a cancer diagnosis</td>
<td>92.0% (3)</td>
<td>91.3% (3)</td>
</tr>
<tr>
<td>%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Making decisions about care</td>
<td>89.4% (4)</td>
<td>89.9% (4)</td>
</tr>
<tr>
<td>%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Talking with medical professionals</td>
<td>87.8% (5)</td>
<td>88.6% (5)</td>
</tr>
<tr>
<td>%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dealing with anxiety / depression</td>
<td>83.7% (6)</td>
<td>85.3% (6)</td>
</tr>
<tr>
<td>%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stress and stress management</td>
<td>87.4% (7)</td>
<td>84.6% (8)</td>
</tr>
<tr>
<td>%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Navigating the health system</td>
<td>87.0% (8)</td>
<td>84.2% (9)</td>
</tr>
<tr>
<td>%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cancer-related fatigue</td>
<td>86.5% (9)</td>
<td>82.9% (12)</td>
</tr>
<tr>
<td>%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nutrition, appetite and weight</td>
<td>86.0% (10)</td>
<td>83.1% (10)</td>
</tr>
</tbody>
</table>

Conclusions: 1) This is the largest survey of patients and caregivers concerning support groups for patients with breast cancer and for a general cancer population. 2) Patients with breast cancer ranked most topics similarly to the general cancer population; however, topics dealing with fatigue and stress management were somewhat more highly ranked by those with breast cancer. 3) Physical, psychological, and communication issues were the most highly ranked issues by breast cancer patients and families. 4) These results should guide the content of support groups to meet the needs of patients and caregivers.

P4-14-04
Parent D, Rallet A, Jovenin N, Yazbek G, Cure H, Rey J-B. Institut Jean Godinot, Reims, France

Background
Few data are available in the literature regarding chemotherapy dosage adjustment in obese patients. Docetaxel is a lipophilic substance, highly bound to albumin; obesity might result in an accumulation of docetaxel and may lead to a higher rate of toxicity. The objective of this study was to assess the tolerance to docetaxel in obese and non obese patients, according to their body mass index (BMI).

Methods
A retrospective study was carried out. Patients were divided according to their BMI, considering a limit of 30 kg/m². All patients received docetaxel (100 mg/m²) as a part of adjuvant chemotherapy for breast carcinoma. Toxicity of docetaxel such as neutropenia, hand-foot syndrome, mucositis, and neuropathy were assessed in both groups. Patients receiving concomitant therapies leading to similar adverse events were excluded (e.g. cyclophosphamide, 5-FU, ...). The included patients received docetaxel alone or in association with trastuzumab or bevacizumab. Qualitative data analysis was performed using Fisher’s exact test. Quantitative data were studied using the Student test.

Results
Among the 49 patients, 18 (37%) presented with a BMI > 30 kg/m². The median age was 53 years-old in the group BMI > 30 and 55 years-old in the group BMI < 30 (p = 0.9383). The median dose intensity delivery was 91% (74-100%) in the group BMI> 30 and 97.7% (75-100%) in the group BMI < 30 (p = 0.06). The median value for albumin was 75 g/L (59-86 g/L) in the BMI > 30 and 71 g/L (56-79 g/L) in the BMI < 30 (p = 0.122). Toxicity in both groups was as follows:

Table 1 - Docetaxel toxicity according to patients’ BMI

<table>
<thead>
<tr>
<th>Neutropenia - n (%)</th>
<th>BMI &gt; 30 kg/m² (n=18)</th>
<th>BMI &lt; 30 kg/m² (n=31)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 (22.2%)</td>
<td>3 (16.6%)</td>
<td>1 (3.2%)</td>
<td>0.12</td>
</tr>
<tr>
<td>Febrile neutropenia - n (%)</td>
<td>1 (22.2%)</td>
<td>1 (3.2%)</td>
<td>0.17</td>
</tr>
<tr>
<td>Hand-foot syndrome - n (%)</td>
<td>8 (44.4%)</td>
<td>1 (3.2%)</td>
<td>0.014*</td>
</tr>
<tr>
<td>All toxicities - n (%)</td>
<td>11 (61.1%)</td>
<td>7 (22.6%)</td>
<td>0.013*</td>
</tr>
</tbody>
</table>

No neuropathy was observed in both groups.

Conclusions
Obese patients (BMI > 30 kg/m²) presented with more toxicities (p = 0.013). Hypoalbuminemia did not represent a risk factor for toxicity in this study as patients had normal albumin levels. Obesity is a risk factor for toxicity in patients treated with docetaxel. Dosage calculation according to body surface area is not suitable for these patients. Toxicity in the group BMI < 30 seems to demonstrate greater susceptibility to the molecule for these patients.

Further studies will try to identify the different mechanisms of toxicity of docetaxel involved obese patients, including the interaction between docetaxel and fats. Measures have also to be taken to obtain a favorable effectiveness/toxicity balance in obese patients.
**P4-14-05**

Chemotherapy-Induced Alopecia, Body Image and Psychological Distress in Women with Breast Cancer: A Prospective Study.

Choi E-K, Kim J-R, Nam S-J, Lee J, Yang J, Lee S-K, Noh D-Y, Han W, Cho J. Samsung Comprehensive Cancer Center, Samsung Medical Center, Sungkyunkwan University School of Medicine; Johns Hopkins Bloomberg School of Public Health; Samsung Medical Center, Sungkyunkwan University School of Medicine; Konkuk University Medical Center, Konkuk University School of Medicine; Seoul National University Hospital, Seoul National University School of Medicine

**Purpose:** While chemotherapy-induced alopecia (CIA) is known to be associated with lower quality of life, the longitudinal effect of alopecia on body image and distress in unknown. This study examined the effect of CIA on body image and psychological distress during active breast cancer treatment.

**Patients and Methods:** Between July and Dec 2010, we recruited patients with non-metastatic breast cancer who were expected to receive adjuvant chemotherapy (N=411) from two cancer hospitals in Seoul, Korea. After excluding 15 patients with recurrence and 65 patients lost to follow-up, the present analysis is based on 331 patients (80.5%). Participants completed questionnaires on body image, quality of life, alopecia distress at enrollment (before surgery), before chemotherapy (2 weeks post surgery), during chemotherapy (3 months post surgery), and after chemotherapy (6 months post surgery). Body image and quality of life were assessed by the EORTC QoL Questionnaire BR23. Alopecia distress was assessed by the Alopecia Distress Scale, which evaluated distress in 4 domains: physical, emotional, daily activity, and relationship.

**Results:** The mean age of the participants was 46.4 (SD 7.91) year. 44.9% and 37.6% of them had stage I and II breast cancer, respectively. 83.0% of the patients had lumpectomy, and 70.6% and 85.9% had chemotherapy and radiotherapy, respectively. Patients who experienced alopecia had a significantly more negative body image and higher distress compared to those who did not experience alopecia. Body image rapidly decreased at 3 months post surgery coinciding with alopecia, and its effects lasted until 6 months post surgery. Moreover, there was a highly statistically significant association between more negative body image and higher alopecia distress after adjusting for other socio-demographic and clinical characteristics. The patterns and changes in body image and distress comparing patients with and without alopecia are reported in Table 1.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>Alopecia (range 21~84)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alopecia distress (range 21~84)</td>
<td>23.8 (8.89)</td>
<td>23.2 (7.34)</td>
<td>0.53</td>
</tr>
<tr>
<td>Mean (SD) at baseline</td>
<td>27.8 (24.00)</td>
<td>20.3 (19.12)</td>
<td>0.28</td>
</tr>
<tr>
<td>Change from baseline to 2 weeks</td>
<td>1.826 (-7.15, 2.22)</td>
<td>1.826 (-7.15, 2.22)</td>
<td>0.46</td>
</tr>
<tr>
<td>Difference in change at 2 weeks</td>
<td>0.044 (-4.73, 6.41)</td>
<td>0.512 (18.53, 1.91)</td>
<td>0.00</td>
</tr>
<tr>
<td>Change from baseline to 6 mo</td>
<td>1.856 (18.53, 19.95)</td>
<td>0.512 (18.53, 19.95)</td>
<td>0.00</td>
</tr>
<tr>
<td>Difference in change at 6 mo</td>
<td>1.856 (18.53, 19.95)</td>
<td>0.512 (18.53, 19.95)</td>
<td>0.00</td>
</tr>
<tr>
<td>Body image (range 0-100)</td>
<td>80.5 (19.12)</td>
<td>80.5 (19.12)</td>
<td>0.28</td>
</tr>
</tbody>
</table>

**Discussion:** Health professionals need to develop clinical pathways and education programs to help women with breast cancer manage alopecia distress and negative body image during active treatment.

**P4-15-01**

High Prevalence of Prospective Memory (PM) Impairment in Early Breast Cancer (EBC) Survivors within 1 Year of Adjuvant Chemotherapy Completion: Novel Findings Concerning Post Chemotherapy Cognitive Effects.

Paquet L, Verma S, Collins B, Song X, Wheatley-Price P, Hopkins S, Segal R, Dent S, Mirsky D, Goel R, Young V, Clemons M, Keller O, Chinneck A, Young R, Bedard M. Carleton University, Ottawa, ON, Canada; The Ottawa Hospital Cancer Centre; Ottawa, ON, Canada; The Ottawa Hospital, Ottawa, ON, Canada

**Background** Numerous studies have demonstrated that EBC survivors report more memory problems than healthy controls. However, evidence of impairment on objective tests of memory remains inconclusive. Past research has focussed exclusively on retrospective memory (remembering information from the past when asked to do so), but the complaints of EBC patients appear better described as “prospective memory” failures defined as forgetting to carry out in the future previously formed intentions. The effective performance of many day-to-day activities relies on PM and its impairment has negative consequences in everyday life. Despite its high ecological relevance, no study has evaluated PM functioning in EBC survivors. Consequently, we assessed the prevalence of PM impairment among EBC survivors in the year following completion of chemotherapy.

**Methods** We undertook a cross sectional quantitative case-control study aiming to recruit 80 patients from the Ottawa Hospital Regional Cancer Centre and 80 matched healthy controls from the community. Patients were within 1 year of having completed a first course of chemotherapy. A standardized test of prospective memory (Memory for Intention Screening Test) was administered to both groups. Following the International Cognition and Cancer Task Force recommendation (2008), impairment was defined as a score that fell one standard deviation below the mean performance of the control group. Standardized measures of depression (CESD), anxiety (STAI) and fatigue (Fact-F) were also completed.

**Results** Data are available on 36 patients and 18 controls. Age was well balanced between the groups (case-control mean age 54y vs. 51y, respectively, p=0.204). Overall PM impairment was observed in 41% of the participants. More importantly, the rate of PM impairment was significantly higher in the EBC group than controls (53% vs 17%; p < 0.005; odds ratio = 5.588; 95% CI=1.376 to 22.7). Multivariate logistic regression showed that PM performance was unaffected by age (p=0.459), depression (p=0.358), anxiety (p=0.512) or fatigue (p=0.595).

**Conclusions** These preliminary findings provide further support for the hypothesis that breast cancer treatment can impair cognitive processes and yield new and important insights into the type of memory problems experienced by EBC survivors. They suggest that a significant proportion of patients exhibit deficits in PM, an aspect of memory involved in effective daily functioning. Our results also suggest that emotional distress and fatigue do not contribute to PM functioning. Further studies in this area should be directed at understanding the severity and duration of PM impairment. Acknowledging and studying this vexing problem in EBC survivors will aid in developing appropriate rehabilitation strategies.
P4-15-02
Clinical and Epidemiological Correlates of Elevated Distress Thermometer Scores in Breast Cancer Patients.

Powers K, Pappas L, Buchmann L, Anderson L, Gauhcy L, Rich A, Agarwal J. University of Utah, Salt Lake City, UT; Huntsman Cancer Institute, Salt Lake City, UT

Objectives: Distress is prevalent in breast cancer patients and can be detrimental to quality of life, performance status, treatment adherence, and satisfaction with medical care. The National Comprehensive Cancer Network (NCCN) developed the Distress Thermometer (DT) as a quick and efficient self-assessment tool for screening distress in cancer patients. While surveys estimate that between 20-40% of patients with cancer have significant levels of distress, fewer than 10% are identified and treated. Given time and monetary constraints, it is important to refine screening criteria to identify patients with elevated risk for distress. In this study, we identify clinical and epidemiological factors that are associated with an increased likelihood of elevated DT scores (≥4 and ≥7) in breast cancer patients.

Methods: We assessed 229 consecutive female patients with the DT at their initial consultation for breast cancer at the Huntsman Cancer Hospital between September 2007 and December 2008. The DT screening tool measures a global level of distress using a visual analogue scale from 0-10 in the shape of a thermometer, with zero identified as “No Distress” and ten labeled as “Extreme Distress.” The DT screening tool also includes a checklist of common emotional, family, physical, practical, and spiritual concerns with instructions for the patient to indicate which of those concerns contributed to the distress they experienced within the past week. We chose a score ≥4 as our cutoff for a positive screen for “distress” and a score ≥7 as our cutoff for a positive screen for “extreme distress/depression” based on previous studies. Variables included in the analyses were: age, employment status, race/ethnicity, personal history of depression, family history of breast cancer, marital status, estrogen and progesterone receptor status, stage of cancer, time since diagnosis, and recurrence. Descriptive statistics and logistic regression models were used to determine associations between DT and patient data.

Results: Emotional and physical concerns were associated with scores ≥4 and scores ≥7. Spiritual concerns were significantly associated with patients reporting scores ≥7. Patients who were non-Caucasian, unemployed, had a prior history of depression, who presented for recurrent disease, or who had been recently diagnosed had a higher likelihood of scores ≥4 and scores ≥7.

Conclusions: The likelihood of scoring ≥4 and ≥7 on the DT screening tool is highest during the first 30 days after receiving a breast cancer diagnosis. Four groups of patients should be targeted for aggressive screening: patients with a prior diagnosis of depression, patients presenting with recurrent disease, unemployed patients, and non-Caucasian patients. Interventions should address physical, emotional and spiritual concerns.

P4-15-03
Patient-Provider Communication and Patient Informational Needs for Breast Reconstruction Post-Mastectomy: Results from a National Survey.


For many women, the complexity of processing and learning about their breast cancer diagnosis is further complicated by decisions to be made about breast reconstruction post-mastectomy. Existing studies suggest that these women are provided with information about breast reconstruction options of differing depth, breadth, and quality, but little is known about how this information is received by the patient as well as the value of this information. Guided by Social Cognitive Theory, the Cancer Support Community (CSC) conducted a national survey in an effort to better understand patients’ information-seeking experiences, patient-provider communication, and knowledge about breast reconstruction. 840 U.S. women with breast cancer (762 eligible for breast reconstruction who were then eligible to answer survey questions pertaining to their experiences) participated in the survey online or by paper-and-pencil at CSC affiliate sites in 2010. In addition to demographics, information about their diagnosis, treatment, and experience with reconstruction, women rated their experience with receiving breast reconstruction information from their healthcare professionals as well as their experience searching for and receiving information about options and realistic expectations for breast reconstruction.

Survey participants came from 46 states, were primarily Caucasian (85%), and the mean age at diagnosis was 48.9 years old. Most women reported that either a plastic surgeon (73.3%) or a breast surgeon (64.0%) had spoken to them about reconstruction, followed by oncologist (34.2%) and surgical oncologist (24.8%). Of women whose healthcare team had spoken with them about reconstruction, 57.1% reported that reconstruction was first discussed at diagnosis. Women reported that this information was somewhat (35.1%) or extremely (55.5%) useful, though they sought additional information elsewhere. Aside from their health care team, most women (60%) sought information about reconstruction from other women with breast cancer and half sought information from the Internet. Participants reported that they would have liked to have had more information prior to reconstruction about a variety of topics, including: how they would look after reconstruction (34.8% endorsing), how they would feel after reconstruction (35.5%), and information about their future breast health (25.9%). Open-ended responses suggest many patients experience gaps in information with regard to establishing realistic expectations about the procedures and outcomes, providing comprehensive information at various stages throughout the process, and across various treatment options.

Though many participants reported satisfaction with how information about reconstruction was provided to them and the quality and scope of this information, responses suggest that there is work to be done with regard to establishing realistic expectations about the procedures and outcomes, providing comprehensive information at various stages throughout the process, and across various treatment options.

P4-16-01
Proteomic Analysis of Patient Plasma Identifies Parathyroid Hormone Related Protein (PThrP12-48) as a Potential Biomarker of Breast Cancer Bone Metastasis.

Washam CL, Byrum SD, Leitzel K, Suhail AM, Lipton A, Suva LJ, Winthrop P. Rockefeller Cancer Institute, University of Arkansas for Medical Sciences, Little Rock, AR; Penn State Hershey Cancer Institute, Penn State Hershey Medical Center, Hershey, PA; Lebanon V4 Medical Center, Lebanon, PA

Background: Bone metastasis is a devastating and often incurable phase of breast cancer progression that significantly compromises patient morbidity and mortality. Despite the well-characterized issues associated with tumor progression, there is currently no reliable method to detect or predict which patients have or are at increased risk for developing bone metastasis. In this study a validated plasma-based proteomic profile for the diagnosis of breast cancer bone metastasis...
was developed and a highly discriminatory protein component of the profile identified as a novel fragment of parathyroid hormone related protein (PTHrP).

Materials and Methods: Plasma samples were collected from a total of 110 breast cancer patients. The training set consisted of 38 breast cancer patients with clinical evidence of bone metastasis and 38 with no evidence of a bone metastasis from time of diagnosis to clinical outcome. The independent validation cohort consisted of 34 breast cancer patients with an unknown bone metastasis classification. The training set was analyzed using surface enhanced laser desorption time-of-flight mass spectrometry (SELDI-TOF MS) and 13 discriminatory protein peaks that contributed to a homogeneous partitioning of the training set (n = 76) in multiple statistical, bioinformatics, and machine learning modeling scenarios were identified and used to construct a RandomForest classifier for the detection of breast cancer bone metastasis (sensitivity: 100%, specificity: 100%).

Results: The diagnostic profile identified was then independently confirmed in a blinded fashion using the validation cohort (sensitivity: 97%; specificity: 82%). Importantly, the most discriminatory protein peak (m/z 4260.92 Da) in the plasma of bone metastasis patients was subsequently identified using specific immunodepletion, trypic peptide mapping and peptide sequencing as a unique proteolytic PTHrP fragment, PTHrP(12-48). Ongoing studies are determining the biogenesis and biological activity of this unique PTHrP peptide.

Discussion: Our discovery of PTHrP(12-48) as a novel plasma biomarker of breast cancer bone metastasis validates our plasma proteomic approach and provides the first direct evidence for the existence of a circulating PTHrP biomarker in breast cancer patient plasma that is associated with bone metastasis.

P4-16-02
Problems with Identifying Bone Metastasis-Specific Genes without Considering Biological Differences between ER-Positive and ER-Negative Breast Cancers.
Hayashi N, Iwamoto T, Qi Y, Niikura N, Santarpia L, Nakamura S, Horiobagyi GN, Puszta I, Symmans F, Ueno NT. The University of Texas MD Anderson Cancer Center; Houston, TX; The University of Texas MD Anderson Cancer Center; Houston, TX; Hospital of Prato and Instituto Toscano Tumori, Prato, Italy; Showa University School of Medicine, Tokyo, Japan

Background: Bone metastasis-specific genes in breast cancer have been reported without considering the significant differences in ER status between bone and non-bone metastases. The aims of this study were to validate genes that had been reported as bone metastasis-specific genes using our data set and to identify bone metastasis-specific genes on the basis of biological differences between ER-positive and ER-negative breast cancers.

Patients and Methods: We used Affymetrix GeneChip arrays to analyze tumor samples obtained from 365 primary invasive breast cancer patients who underwent surgery from 1999 to 2008. We excluded patients with HER2-positive breast cancer (normalized HER2 mRNA expression [probe set 216836_s_at] > 12.54). We classified the samples into 3 cohorts according to first metastatic site: bone, non-bone, or no metastasis. Differential expression of genes between bone and non-bone cohorts that were differentially expressed was identified using the Cox proportional hazards model, and gene sets were assessed using gene-set analysis.

Results: Of the 365 patients, 34 (9.3%) were included in the bone cohort and 32 (8.8%) in the non-bone cohort. Two hundred fourteen (58.6%) had ER-positive and 151 (41.4%) had ER-negative breast cancer. First, we performed gene-set analysis using 5 gene sets that had been reported to be associated with bone metastasis. One gene set, which had been detected using an ER-negative breast cancer cell line, was validated as predicting bone metastasis in ER-positive breast cancer. None of the 5 gene sets predicted bone metastasis in ER-negative breast cancer. We then determined the levels of individual genes associated with bone metastasis by ER status using all 16,712 probe sets filtered by average gene expression level. When we analyzed all patients without any stratification by ER status, as in previous studies, 592 probe sets were significantly overexpressed in the bone cohort compared with the non-bone cohort, with a false discovery rate of ≤0.05. However, when we analyzed ER-positive and ER-negative breast cancers separately, no genes were found with significant differences between bone and non-bone cohorts. Finally, we used 2,246 functionally annotated gene sets assembled from Gene Ontology to examine possible biological differences between bone and non-bone cohorts. In the bone cohort, 151 and 125 gene sets were significantly overexpressed in ER-positive and ER-negative breast cancers, respectively (P ≤ 0.05). Ppathways related to Cellular growth and proliferation, intracellular and second-messenger signaling were overexpressed in ER-positive breast cancer, whereas pathways related to nuclear receptor and cytokine signaling were overexpressed in ER-negative breast cancer. Most bone-metastasis-related pathways were different in ER-positive and ER-negative breast cancers (91.4% and 89.6% of the gene sets, respectively).

Discussion: No genes were found that can predict bone metastasis. ER-positive and ER-negative breast cancers have different biological potentials for bone metastasis. Therefore, we need to assess the prediction model of bone metastasis based on the biological features for each ER status separately.

P4-16-03
Proof of the Anti-Tumour Effect of Zoledronic Acid (ZA) in Naive Bone-Only Metastatic and Locally Advanced Breast Cancer: Results from the Biological Window Therapy.
Foroni C, Andreis D, Maldotti M, Cappelletti MR, Generali DG. Istituti Ospitalieri di Cremona, Cremona, Italy

Background: Pre-clinical studies have demonstrated an anti-tumour activity of ZA in several cancers. However, the clinical evidence of the ZA anti-tumour effect is still uncertain. Our study aimed to prove that ZA has anti-tumour activity administered alone as a biological window of 14 days therapy on naïve bone-only metastatic and locally advanced breast cancer.

Material and Methods: 27 patients with locally advanced breast cancer (Group 1) and 12 patients at their first relapse with bone metastasis only (Group 2) received 4 mg single dose of ZA before starting with any treatment. In Group 1, Ki67 expression was evaluated in tumour specimens obtained before and after ZA administration (basal, day 14). In Group 2, circulating tumour cells (CTCs) were evaluated at baseline, 48 hrs and day 14. In Group 1 and 2, the apoptosis and necrosis of tumour cells were tested on blood samples by M30 and M65 ELISA assay respectively. Parallel, the anti-antitumor activity administered alone as a biological window of 14 days therapy on naïve bone-only metastatic and locally advanced breast cancer.

Results: A significant reduction of CTC number after 48 hrs (median, 16 to 7; p=0.045), followed by a tendency to increase on
day 14 (p=0.093). The M30/M65 analysis performed on paired blood samples in Group 2 showed after 14 days a significant increase in M65 (median, 97 to 112 U/L; p=0.018), more accentuated than the concomitant increase in M30 (p=0.308). In MDA-MB-231 bearing mice, ZA treatment caused a significant inhibition of tumor growth. Conclusions: These results are the first prospective clinical data showing a direct anti-tumour effect (either on the tumour or on CTCs) of ZA, confirming the in vitro data. By directly affecting the proliferation, ZA could have an anti-tumour effect via induction of necrosis, suggesting its possible use in combination with conventional oncologic treatments of breast cancer.

**P4-16-04**

**An Open-Label, Phase IIa, Non-Randomized Study of Radium-223 in Breast Cancer Patients with Bone Dominant Disease No Longer Considered Suitable for Endocrine Therapy.**

Coleman R, Flamen P, Naume B, Jerusalem G, Garcia C, Piccart M, O’Bryan-Tear CG, Aksnes A-K. Weston Park Hospital, Sheffield, United Kingdom; Institut Jules Bordet, Brussels, Belgium; Oslo University Hospital, Oslo, Norway; CHU Sart Tilman, B35, Liege, Belgium; Algea ASA, Oslo, Norway; Bayer Healthcare Pharmaceuticals, Montville, NJ

**Background:** Radium-223 (Alpharadin™) is a 1st-in-class alpha-pharmaceutical. It targets bone metastases (mets) with high-energy alpha-radiation of extremely short range that spares bone marrow. These characteristics generate highly localized radiation zones that may inhibit tumor progression and induce pain relief. Radium-223 has a profound effect on markers of bone metabolism and has shown a survival advantage over standard of care in pts with castration-resistant prostate cancer. The study’s main objective was to investigate whether multiple IV injections (inj) of radium-223 have any clinically relevant effect on bone markers in metastatic breast cancer (MBC) pts with bone dominant disease (BDD). **Methods:** Study included MBC pts with BDD who had progressed (based on imaging or other clinically relevant information) on endocrine therapy and were no longer considered suitable for endocrine therapy. In this open-label, multicenter, single-arm, phase IIa study (EudraCT #2009-012189-30), 23 pts were scheduled to receive 4 IV inj of radium-223 50 kBq/kg every 4 wk. Bone markers were assessed at baseline, prior to every treatment, and thereafter at each follow-up visit. Primary efficacy endpoint was change in urine levels of NTX (uNTX) and bALP at 16 wk. Functional imaging with FDG-PET was performed in 20 pts at baseline and wk 8 and 16. Symptomatic response was assessed using validated questionnaires. **Results:** Histologic types were ductal carcinoma (n=12), lobular carcinoma (n=7), and others (n=2). Median interval from diagnosis of breast cancer to 1st relapse was 3 (1, 11) years. All except 1 pt received endocrine therapies, and adjuvant chemotherapy was given to approximately 60%. 21/23 pts were treated concurrently with bisphosphonates. 15 of 23 pts received all 4 inj of radium-223; 4/23 received 3 inj; and 4/23 received 2 inj. Pts who received ≤4 inj had disease progression (bone and visceral mets [n=3], visceral mets [n=3]) or withdrawn consent (n=1) and were not eligible to continue radium-223. One pt died before 4th inj due to atrial fibrillation. Median age was 58 (41, 83) years. Median uNTX levels were reduced by 20% (from 36 to 29 nmolBCE/mmol creatinine; \(P=0.03\)) and 5% (from 23 to 22 nmolBCE/mmol creatinine; \(P=NS\)) at wk 8 and 16 respectively; 12/23 and 9/13 pts (for whom data were available) had a decrease in uNTX at wk 8 and 16 respectively. Median bALP levels were reduced by 33% (from 22.1 to 12.1 ng/mL; \(P=0.0001\)) at wk 8 and 33% (from 22.1 to 10.4 ng/mL; \(P=0.04\)) at wk 16. Bone-ALP levels were reduced in 20/22 pts at wk 8 and in 10/12 pts (for whom data were available) at wk 16. Radium-223 was found to be safe and well tolerated. 3 pts had serious AEs, none related to study drug; 1 of them died due to disease progression. Functional imaging results, additional bone marker data, and pt-reported outcomes are being analyzed. **Conclusions:** Radium-223 consistently reduced uNTX and bALP during the 16-wk treatment period, results observed in addition to bisphosphonate use. Results show radium-223 targets the areas of increased bone metabolism caused by bone mets. Radium-223 found to be safe and well tolerated, confirming highly tolerable SE profile seen in other studies.

**P4-16-05**

**A Randomized Phase 2 Study of a Loading Dose of Ibandronate in Patients with Bone Metastases from Breast Cancer.**

Ritchie DM, Bray C, Canney P. Beatson West of Scotland Cancer Centre, Glasgow, United Kingdom

Bisphosphonates have proven efficacy in reducing skeletal complications in metastatic breast cancer. Potent third generation bisphosphonates are more effective but the dose limiting toxicity is usually renal. Ibandronate is a 3rd generation bisphosphonate available in oral and intravenous form which offers many clinical advantages in ease of administration and lack of renal toxicity. A dose-response effect has been observed between 2mg and 6 mg IV doses and there is a single study suggesting that higher dosing (4 mg IV daily for 3 days) is tolerable and effective. A steady state of oral ibandronate is achieved at 8 days with oral administration but time to response is not known. In trials so far, IV ibandronate 6mg appears safe. Due to lack of renal toxicity there is potential for further escalation of IV ibandronate. The objective of this study was to establish if an IV loading dose can improve efficacy and time to biochemical response compared to oral standard therapy and to assess the safety of a higher IV dose of ibandronate.

**Methods and Patients:** This was an open randomised phase II study conducted on patients with bone metastases from breast cancer comparing IV ibandronate 12 mg on day 1 followed by oral ibandronate 50 mgs daily (Arm A) with standard oral therapy of 50 mgs daily from day 1 (Arm B). The primary study end-point was the percentage reduction in serum CTX from baseline by day 5 on study, secondary end-points were the percentage reduction of bone turnover markers including serum CTX from baseline end of week 8 and percentage reduction in urine NTX from baseline to day 5 on study and baseline to end of week 1-8. Bone pain was recorded by Brief Pain Inventory. Patients had metastatic breast cancer with proven bone metastases, no previous treatment with bisphosphonates or other bone directed therapy within 6 months and no change in systemic therapy within a 3 months preceding trial therapy. Sample size of 22 patients in each arm was calculated to give a 90% chance of detecting a 20% difference in average percentage reduction between the IV and oral arms.

**Results:** Seventeen patients were randomised to each study arm. A more rapid change in bone turnover markers was demonstrated in patients receiving the 12 mg loading dose of ibandronate. There was a 15.8% greater reduction of serum CTX in Arm A compared with Arm B. The primary study endpoint was the percentage reduction in serum CTX from baseline by day 5 on study, secondary end-points were the percentage reduction of bone turnover markers including serum CTX from baseline end of week 8 and percentage reduction in urine NTX from baseline to day 5 on study and baseline to end of week 1-8. Bone pain was recorded by Brief Pain Inventory. Patients had metastatic breast cancer with proven bone metastases, no previous treatment with bisphosphonates or other bone directed therapy within 6 months and no change in systemic therapy within a 3 months preceding trial therapy. Sample size of 22 patients in each arm was calculated to give a 90% chance of detecting a 20% difference in average percentage reduction between the IV and oral arms.

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12 mg of IV ibandronate and no evidence of additional renal toxicity. Conclusion: A 12 mg dose of IV ibandronate can be safely administered without additional renal toxicity. A rapid reduction in bone turnover markers is demonstrated within 5 days of IV loading dose of ibandronate. Potential exists for dose escalation of ibandronate. The clinical benefit of a more rapid reduction in bone turnover markers is unknown.

P4-16-06
Expression Patterns of Receptor Activator of Nuclear Factor-κB (RANK) and Src in a Series of Primary Breast Tumors (BT) and Bone Metastases (BM) in Patients (pts) with Metastatic Breast Cancer (MBC).
Gcapal A, Comen E, Redana S, Evangelista L, Giri DD, Zhang XH, Patil S, Akram M, Norton L, Hudis CA, Fornier MN. Memorial Sloan-Kettering Cancer Center, New York, NY; Sloan-Kettering Institute, New York, NY; Piemontese per l’Oncologia - Institute for Cancer Research and Treatment, Candiolo, Italy; Istituto Oncologico Veneto (HOV - IRCCS), Padua, Italy

Background: BM develops in 65-70% of pts with MBC. RANK and its ligand (RANK-L) can be critical in the development and progression of BM. Src overexpression and deregulation occurs in many solid tumors but it has not been fully characterized although an association between Src activity defined by a gene expression signature and BM particularly in ER+ pts has been described. (Zhang XH et al. Cancer Cell. 2009) Our goal was to elucidate the relationship between Src and RANK expression in BT and BM in relation to estrogen-/progesterone-receptor (ER/PR)/HER2 status of BT and BM. Scoring: 0=negative, 1+=weak, 2+=intermediate, 3+=strong and the percent of positive tumor cells; RANK+ = 2-3+, > 1% of cells; Src+ = 1-3+; > 1% of cells. Associations between RANK/Src expression and tumor histology (invasive ductal carcinoma (IDC) vs invasive lobular carcinoma (ILC)).

Methods: Immunohistochemistry (IHC) for RANK (R&D Systems clone 80707) and Src (Cell Applications Inc. Phospho Tyr-416) protein expression was performed on archived paraffin embedded BT and BM. Scoring: 0=negative, 1+=weak, 2+=intermediate, 3+=strong and the percent of positive tumor cells; RANK+ = 2-3+, > 1% of cells; Src+ = 1-3+; > 1% of cells. Associations between RANK/Src expression and tumor characteristics were assessed using the chi-square test or McNemar’s test for pairs, as appropriate.

Results: From the MSKCC database, using an IRB-approved waiver of consent, we identified 54 pts with MBC who underwent surgical biopsy of a metastatic bone lesion at our center between 2005-2010, and had tissue available for further testing. 17 corresponding BT samples were identified. At the time of diagnosis, 43 (79.5%) primary tumors were ER or PR (+); 6 (11%) were HER2+; 41 (76%) were invasive ductal carcinoma. 87% of BM expressed RANK and 44% expressed Src. (Table 1) No significant correlation between RANK or Src expression in BM and ER/PR/HER2 status of BT was observed. A significant correlation between RANK expression and BT histology was observed, (p=0.0016): 93% of IDC were RANK (+), in comparison to 50% of invasive lobular carcinomas. RANK expression was not significantly different between primary tumor and metastatic bone samples (p=0.06). There was a borderline significant difference in Src expression between primary and metastatic site (p=0.06).

Table 1. Tumor Characteristics (n = 54)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Number of patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER status</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>43 (79.5)</td>
</tr>
<tr>
<td>Negative</td>
<td>10 (18.5)</td>
</tr>
<tr>
<td>Unknown</td>
<td>1 (2)</td>
</tr>
<tr>
<td>PR status</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>40 (74)</td>
</tr>
<tr>
<td>Negative</td>
<td>13 (24)</td>
</tr>
<tr>
<td>Unknown</td>
<td>1 (2)</td>
</tr>
<tr>
<td>HER2 status</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>6 (11)</td>
</tr>
<tr>
<td>Negative</td>
<td>46 (85)</td>
</tr>
<tr>
<td>Unknown</td>
<td>2 (4)</td>
</tr>
<tr>
<td>Histology</td>
<td></td>
</tr>
<tr>
<td>IDC</td>
<td>41 (76)</td>
</tr>
<tr>
<td>ILC</td>
<td>8 (15)</td>
</tr>
<tr>
<td>Mixed IDC/ILC</td>
<td>4 (7)</td>
</tr>
<tr>
<td>Other</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Bone Metastases</td>
<td></td>
</tr>
<tr>
<td>RANK expression</td>
<td>47 (87)</td>
</tr>
<tr>
<td>Src expression</td>
<td>24 (44)</td>
</tr>
<tr>
<td>Primary Tumor</td>
<td></td>
</tr>
<tr>
<td>RANK expression</td>
<td>16 (94)</td>
</tr>
<tr>
<td>Src expression</td>
<td>12 (71)</td>
</tr>
</tbody>
</table>

Conclusions: In our cohort, no correlation between RANK or Src by IHC and ER/PR/HER2 was identified but RANK expression was more common in IDC than ILC. Fidelity was high for RANK between primary and metastatic lesions while Src expression may possibly vary.

P4-16-07
Mitchell ME, Taylor A, Lowe KA, Langeberg W, Alexander D, Kelsh M, Body J-J. Exponent; Amgen; CHU Brugmann

Background: Bone metastases are common in breast cancer patients and an important cause of morbidity and mortality. This review provides the first systematic and quantitative summary of the proportion of breast cancer patients with bone metastasis and their survival, presented by stage at diagnosis and length of follow-up.

Materials and methods: A literature review was conducted using PubMed to identify full-text studies published in English 1999-2009. Studies were included if they had ≥100 adult patients with Stage I-IV breast cancer and were generalizable to the overall breast cancer population. The design and intent of included studies varied, including clinical trials of bone-targeted therapies and observational studies of cancer patients evaluating staging techniques, biomarkers, and other factors. To improve homogeneity, studies were grouped by stage at diagnosis, whether follow-up for bone metastases occurred, and the clinical point at which the proportion of patients with bone metastasis was estimated (e.g., at initial staging). Studies reporting survival were only included in quantitative summaries if survival was measured from the date of bone metastasis diagnosis. With regard to clinical trials of bisphosphonates, only proportion and survival data from the placebo group were utilized since the goal was to describe bone metastasis among patients untreated with bone-targeted therapy. Meta-analytic techniques were applied to (1) proportion data using random effects models to calculate weighted averages, and (2) survival data using an average of reported median survivals. Various sensitivity analyses were also conducted.

Results: Nineteen studies were included in our quantitative summary of proportion data. At initial staging of breast cancer, 7% (N=2; 95% confidence interval [CI]=5-9%) of all patients had bone metastases and 69% (N=2; 95% CI=47-85%) of Stage IV patients had bone metastases. Among breast cancer patients followed from the diagnosis of Stage I-III breast cancer, 10% (N=6; 95% CI=6-15%) had bone metastases after a median follow-up <10 years, compared to 24% (N=3; 95% CI=19-31%) after a median follow-up ≥10 years. Twelve studies were included in our quantitative summary of survival data.
Survival was longer among patients with metastases solely in bone (N=7; average median=30 months), compared to patients with bone metastases and metastases at other sites (N=5; 23 months) and patients with metastases only at sites other than bone (N=3; 9 months).

**Discussion:** Significant heterogeneity across studies was observed, reflecting the variability in study populations, locations, and other factors. Despite this and other limitations (e.g., few studies in some categories), this represents the first analysis of the literature to quantify the prevalence and survival of bone metastasis in breast cancer. Our results indicated that bone is a site of metastasis among more than 50% of Stage IV patients at diagnosis of breast cancer. Median survival among breast cancer patients with metastases located solely in the bone is at least six months longer compared to those with metastases at multiple sites (including bone) and sites other than bone.

**P4-16-08**

**Pilot Randomized Trial of De-escalated (q12 Weekly) Versus Standard (q3-4 Weekly) Intravenous Bisphosphonates in Women with Low-Risk Bone Metastases from Breast Cancer.**

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**Background:** Bone-targeted agent such as bisphosphonates (BPs) can reduce skeletal complications from bone metastases but have no effect on either disease progression or survival. Despite substantial variability in the frequency and severity of skeletal complications, patients are empirically treated with BPs at the same dose and schedule irrespective of their individual risk.

**Materials and Methods:** A pilot, randomized, non-inferiority trial was conducted. Patients with low-risk bone metastases (serum C-telopeptide [CTx] <600ng/L after at least 3 cycles of monthly IV BP) were assigned to pamidronate 90mg IV either every 3-4 weeks (control) or every 12 weeks (de-escalated). Data on serum CTx and bone alkaline phosphatase (BAP), pain scores (brief pain inventory [BPI] and functional assessment of cancer therapy-bone pain [FACT-BP]) were collected at 12 weekly intervals for 48 weeks.

**Results:** Fifty-four patients were approached, 44 provided consent and 38 were eligible for randomization. Median age was 55 (range 29-77) and mean baseline CTx was 319ng/L (range 10-526). Thirty-five participants (92%) completed the trial, 2 withdrew consent and one participant died. Fourteen control group participants (73.7%) and 13 experimental group participants (68.4%) maintained CTx in the low risk range (test for two proportions p=0.64). All patients not maintaining CTx in the low risk range showed evidence of both visceral and bone progression. Compared to the control group, there was a trend towards increasing CTx with time in the experimental group (p=0.10). There was no significant difference in BAP (p=0.37), BPI (p=0.21) or FACT-BP scores (p=0.59) between the two groups. Over the 48 week follow-up, two skeletal events were observed in each group.

**Conclusions:** Randomized trials of de-escalated BP therapy in women with low-risk bone metastases are feasible. Twelve-weekly pamidronate appears non-inferior to 3-4 weekly treatment. Larger trials are required to assess whether: 1) increasing CTx levels with de-escalated therapy will lead to higher rates of skeletal complications and 2) whether BP should be given every 3-4 weeks in patients with progressive visceral and bone disease.

**P4-16-09**

**Health Resource Utilization (HRU) Associated with Skeletal-Related Events (SREs) in Advanced Breast Cancer Patients with Bone Metastases: Results from a Prospective Multinational Observational Study.**

Läffner D, Lorusso V, Duran I, Hechmati G, Garzon-Rodriguez C, Aschroft J, Bahl A, Ghelani P, Wei R, Thomas E, Hoefeler H. Universitätsmedizin Berlin, Berlin, Germany; Oncology Institute ASL, Lecce, Italy; Centro Integral Oncologico Clara Campal (CIOCC), Madrid, Spain; Ament (Europe) GmbH, Zug, Switzerland; Instituto Catalán Oncología ICO-IDIBELL, Barcelona, Spain; Pinderfields General Hospital, Wakefield, United Kingdom; University Hospitals Bristol, Bristol, United Kingdom; Ovatech Solutions, London, United Kingdom; Amgen Inc., So. San Francisco, CA; Forschungszentrum Ruhr, Witten, Germany

**Background:** Patients with advanced breast cancer and bone metastases suffer from skeletal complications (SREs), defined as spinal cord compression [SCC], surgery to bone [SB], pathologic fracture [PF] or radiation to bone [RB]). Planning future resource requirements and estimating the value of new treatment options requires prospective data on the health resource burden. However, there is a lack of these data in the literature.

**Materials and Methods:** Patients had bone metastases secondary to advanced breast cancer and were eligible to be included in the study if they had: at least one SRE within 90 days prior to enrolment; life expectancy >6 months; ECOG ≤2. HRU (number and length of inpatient hospitalizations, outpatient visits, emergency room visits, number of procedures, etc) associated with SREs was collected retrospectively for 90 days prior to enrolment and prospectively for up to 18-21 months. Attribution of HRU to each SRE was determined independently by the investigators. This pooled European analysis includes data for breast cancer patients from centers in Germany, Italy, Spain and UK.

**Results:** A total of 223 eligible patients with breast cancer and bone metastases were enrolled from the four countries. A total of 118 of 457 SREs (25.8%) were associated with inpatient stays with a mean duration of 18.2 (SD=15.7) days per inpatient stay (for the total 125 stays; a single SRE could contribute multiple hospitalizations). The length of inpatient stays varied by facility (i.e., oncology, radiation, surgical) and SRE type. The most common SRE requiring hospitalization was SB (42 of 54 events [77.8%]) with 45 inpatient stays requiring an average length of stay of 15.1 (SD=16.8) days. The least common SRE requiring hospitalization, RB (27 of 279 events [9.7%]), was still associated with 23 inpatient stays with an average of 16.7 (SD=12.4) days per inpatient stay. A total of 342 SREs (74.8%) required an outpatient visit and 159 (34.8%) required >5 visits. As expected, RB was associated with the highest number of outpatient visits (239 of 279 [85.7%] SREs). SB and PF were associated with fewer outpatient visits with 23 of 54 (42.6%) of 54 SREs and 66 of 105 (62.9%) SREs requiring a visit, respectively. 22 of 457 (4.8%) SREs were associated with an emergency room visit.

**Discussion:** SREs can lead to lengthy hospitalizations and numerous outpatient visits. Neither pain requiring opioid use nor changes in cancer therapy to treat bone pain were reported as SREs, although they may have led to additional inpatient and outpatient visits. Thus, HRU estimated in this study likely underestimates overall HRU associated with SREs in advanced breast cancer patients. Relatively low utilization of emergency room visits reported here may be due to emergency care provided directly by the specialist oncology unit or the patient visiting...
P4-17-01
Trastuzumab Does Not Increase the Incidence of Central Nervous System (CNS) Relapses in HER2-Positive Early Breast Cancer: The HERA Trial Experience.

Pestalozzi B, Holmes E, Metzger O, de Azambuja E, Hogge L, Scullion M, Gelber R, Piccart-Gebhart M, Cameron D. HERA Study Team

Background: Retrospective studies of HER2-positive metastatic breast cancer (BC) showed an incidence of CNS metastases of 21% to 34%. We investigated the incidence and clinical aspects of CNS relapse (CNS-R) in patients (pts) enrolled in the HERA trial, a prospectively randomized adjuvant trial in node + or high-risk node - HER2-positive early BC pts.

Methods: 3401 pts were randomized into the 1-year trastuzumab (1yT) and the observation (obs) arms of HERA (Piccart-Gebhart et al, 2005, Gianni et al, 2011). The cumulative incidences of first disease-free survival (DFS) events in the CNS vs other sites were estimated using competing risk analysis. The database of the main study had a clinical cut-off date of 9th June 2008. To obtain additional information regarding CNS-R (including occurrence of CNS-R after first DFS event), a specific CNS-directed questionnaire was sent to investigators of pts who were deceased as of July 2009. Information collected included the date of CNS-R, whether it was symptomatic, the type of CNS-R (brain metastases (BM) or meningeal carcinomatosis (MC)), methods of diagnosis, and treatments at the time of CNS-R.

Results: 1yT significantly reduced the risk of other DFS events (p=0.000017, Gray’s test), but not of CNS-R (p=0.55) as first event (see table). During the first year of follow up, CNS-R accounted for 15 (14.9%) of the 101 first DFS events in the 1yT arm and 15 (7.7%) of the 194 first DFS events in the obs arm. The analysis of baseline patient and tumor characteristics associated with CNS-R as first event confirmed known risk factors such as young age (<35y), T3 tumor, ≥ 4 + LN, ER neg, and G3.

Based on the survey data, CNS-R was symptomatic in 189 pts (87.1%) with no differences between arms. BM were present in 211 pts (97.2%), absent in 5 (2.3%), and missing information in 1 (0.5%). MC was diagnosed in 25 pts (11.5%), absent in 187 (86.2%), missing information in 5 (2.3%). Frequencies for BM and MC were very similar in both arms.

Conclusion: This retrospective analysis of a prospective large study shows more than 50% incidence of clinically diagnosed CNS-R in HER2-positive BC pts who have died. CNS-R was symptomatic in most pts. CNS-R at any time was less frequent in the 1yT arm (88 vs 129). There is no evidence that adjuvant trastuzumab increases the incidence of CNS-R.

P4-17-02
Whole-Brain Radiation Therapy Plus Concomitant Temozolomide for the Treatment of Brain Metastases from Breast Cancer: A Randomized Prospective Multicenter Phase II Study.


BACKGROUND: A previously published study of temozolomide (TMZ) concurrent with whole-brain radiation therapy (WBRT) reported significant improvement in response rates and a nonsignificant trend toward improved overall survival compared with WBRT alone in patients with brain metastases in different type of tumors. A prospective randomized phase II trial of concurrent WBRT and TMZ versus WBRT alone was conducted to assess whether the concurrent use of WBRT-TMZ resulted in measurable radiological response differences at 6 weeks after the treatment in homogeneous population of breast cancer (BC) patients.

PATIENTS AND METHODS: In this intent-to-treat study, patients with metastatic breast cancer to the brain and not suitable for surgery or radio-surgery were enrolled in a randomized prospective multicenter phase II study between February 2008 and December 2010. Patients received temozolomide 75 mg/m²/2day (days 1-15) concurrently with WBRT 30 Gy/10 fractions. Patients were stratified by type and metastatic sites. The (MRI) response at 6 weeks was the main criteria; response was defined as complete (CR) or partial response (PR). Initial and follow-up MRI were realized on a 1.5T unit (Siemens) by axial SE-T1 WIs and FLAIR WIs with GdDTPA injection, EG-3DT1 WIs after GdDTPA. Five target lesions (over 10 mm greatest diameter) were measured in their 3 dimensions. Non measurable lesions were noticed. The treatment tolerance and clinical responses were also evaluated. The study was designed according to a modified Fleming two-stage plan based on response hypotheses of 30% (H0) versus 50% (H1). Twenty or more responses among 50 subjects were needed to declare the treatment effective, with risk errors alpha 9% and beta 7%.

RESULTS: One hundred metastatic breast cancer patients were enrolled in the study, as following: 49 in WBRT-TMZ and 51 in WBRT groups. The median age was 55 years [range, 29-79]. The median follow-up was 12 weeks [range, 1-94]. There were 15/49 (31%) partial responses (PR) in WBRT-TMZ and 18/51 (35%) PR in WBRT group. No complete responses were observed. The symptoms relief was obtained in 27/49 and 31/51 pts, respectively. The treatment tolerance was acceptable in both groups.

CONCLUSION: The benefit of adding TMZ to WBRT was not confirmed at 6 weeks. The tolerance was good in both groups. Longer follow-up is needed to evaluate the combination of TMZ with WBRT in terms of progression free survival.
**P4-17-03**
Incidence Rate of Asymptomatic Brain Metastases in Patients with HER2+ Metastatic Breast Cancer Screened for EGF111438/CEREBEL Study.
Pivot X, Hackmann J, Manikhias A, Moore Y, Parikh R, Kohli D, Joshi A, Akton G, Coleman R. University Hospital of Besancon; Marien-Hospital; St Petersburg City Clinical Oncology Dispensary; GlaxoSmithKline, Collegeville, PA; GlaxoSmithKline, Uxbridge, United Kingdom; Weston Park Hospital

**BACKGROUND**
Brain metastases constitute a major clinical concern occurring in 28-43% of women treated for HER2+ metastatic breast cancer (MBC). The initial diagnosis of Central Nervous System (CNS) lesion is commonly based upon development of neurological symptoms. Data to address incidence of CNS lesions in asymptomatic patients are rare and not generally reported. EGF111438/CEREBEL study requires systematic screening for brain metastases by brain MRI and the ineligible patients due to detection of asymptomatic brain metastases are reported here.

**METHODS**
The patients screened for CEREBEL have consented to a multi-centre, open-label Phase III study in subjects with HER2+ metastatic breast cancer who received prior treatment with anthracyclines or taxanes and trastuzumab in the adjuvant or metastatic setting. Eligible subjects must not have any history of CNS metastases (including leptomeningeal involvement) or have evidence of benign or malignant brain tumours and/or clinical evidence of spinal cord metastases at baseline. In order to confirm this, subjects must have negative brain MRI at baseline as confirmed by independent review (IR). All MRI scans of the brain had to be acquired with gadolinium contrast agent (T1 only) and 3mm slice thickness without gaps. The primary endpoint of CEREBEL is the incidence of CNS lesions as first site of relapse. A total of 650 subjects (325 per group) are expected to be randomized in the study who were clinically thought to be free of brain lesions actually had brain metastases verified by brain MRI. We therefore conclude that screening for occult brain metastases by MRI is important for trials with defined CNS endpoints.

**RESULTS**
The study is currently recruiting with 322 patients enrolled as of 15.06.2011. At this date, the total number of patients screened were 492 and 170 of these patients were screen failures. There are 15 patients in screening. The percentage of screen failures due to asymptomatic brain metastases assessed by investigators was 13.5% (23/170). The overall incidence of clinically asymptomatic brain metastases assessed by investigators for this study is 4.7% (23/492).

**CONCLUSIONS**
Around 5% of all screened patients (14% of screen failures) in this study who were clinically thought to be free of brain lesions actually had brain metastases verified by brain MRI. We therefore conclude that screening for occult brain metastases by MRI is important for trials with defined CNS endpoints.

**P4-17-04**
Pharmacokinetic Disposition of PEGylated Liposomal Doxorubicin Compared with Non-Liposomal Doxorubicin in an Intracranial Breast Cancer Murine Model.
Anders CK, Adamo B, Walsh MD, Karginova O, Darr D, Deal AM, Santos C, Bash R, Hanna SK, Carey LA, Miller CR, Sharpless N, Zamboni WC. University of North Carolina at Chapel Hill (UNC), Chapel Hill, NC; UNC Lineberger Comprehensive Cancer Center, Chapel Hill, NC; Lineberger Comprehensive Cancer Center, Chapel Hill, NC; University of Messina, Messina, Italy

**Background:** Breast cancer brain metastases are an increasingly common consequence of advanced breast cancer associated with poor prognosis and survival. Systemic therapies capable of crossing the blood brain barrier to control both intra- and extracranial disease are few. Nanoparticle agents, such as liposomes, achieve a higher concentration and prolonged exposure in solid tumors. Liposomal formulations of anti-cancer agents have also been shown in preclinical and clinical studies to enhance delivery to tumors within the brain. We compared the pharmacokinetic (PK) disposition of PEGylated liposomal doxorubicin (Doxil®; PLD) with non-liposomal doxorubicin (NLD) in an intracranial breast cancer model.

**Methods:** Athymic (nu/nu) mice (~8wks) were inoculated with MDA-MB-231-BR (brain subclone) cells expressing luciferase (2×10^6 cells/ injection) in 5% methylcellulose (5µL) into the right caudate nucleus. On day 18, tumor-bearing mice via bioluminescence imaging were administered PLD or NLD at 6 mg/kg IV x 1 via tail vein. Mice (n=3) were sacrificed at 6 and 24h for PLD and NLD; and 72h for PLD. Plasma and intracranial tumor were processed to measure sum total doxorubicin via high-performance liquid chromatography (HPLC, lower limit of quantification=10 ng/mL). T-tests were used to compare means between treatment groups.

**Results:** Treatment with PLD resulted in higher plasma sum total doxorubicin concentrations compared with NLD (Table). PLD was detected at 72h in plasma. There were higher intracranial tumor sum total doxorubicin concentrations at 6 and 24h for PLD compared with NLD; NLD was below limit of quantification (BLQ) at both time points. Consistent with the plasma PK of PLD, intracranial tumor total doxorubicin concentrations for PLD remained elevated at 72h.

**Conclusions:** Liposomal formulations of anti-cancer agents represent a promising treatment strategy to treat aggressive breast cancer brain metastases due to extended circulation time in plasma and higher exposure in intracranial tumor compared with small molecule counterparts. In this proof-of-concept preclinical study, PLD exhibits both higher and prolonged plasma and intracranial tumor exposure compared with NLD. The mechanism of higher intracranial tumor exposure with liposomal agents is unknown, however may be due to prolonged exposure in plasma, diffusion of encapsulated and/or released drug across the blood brain barrier, or enhanced permeation and retention effect in tumors. The improved PK profile and tumor distribution of PLD compared with NLD in this intracranial BC model are hypothesized to translate to improved efficacy and toxicity profiles over that of non-liposomal formulations and may represent a promising treatment strategy for a disease with limited treatment options.

**P4-17-05**
Brain Metastasis Free Survival (BMFS) Differs between Breast Cancer Subtypes.
Bartsch R, Berghoff A, Bago-Horvat Z, DeVries C, Dubsky P, Pluschnig U, Rudas M, Rottenfusser A, Fitzal F, Dieckmann K, Mader RM, Grant M, Zielinski CC, Steger GG. Medical University of Vienna, Vienna, Austria

**BACKGROUND**
Brain metastases (BM) are frequently diagnosed in patients (pts) with Her2-positive metastatic breast cancer (BC); a rising incidence was also reported in triple-negative disease. We hypothesized that pts with triple-negative or Her2-positive tumours had shorter BMFS as compared to other BC subtypes.
Therefore, we aimed to compare BMFS in pts with Her2-positive, estrogen receptor (ER) positive and triple-negative BC treated at the Medical University of Vienna from 1999-2009. In Her2-positive tumours, we further investigated the influence of ER co-expression on BMFS, as Her2-positive / ER-positive tumours were reported to express less aggressive biological properties.

**METHODS**

BMFS was defined as primary study endpoint and measured as the interval from diagnosis of metastatic BC until diagnosis of BM. A total of 168 pts were identified from a breast cancer database. 34 pts were excluded from this analysis as brain was the first site of disease progression; hence complete datasets from 134 pts were available (69 Her2-positive; 33 triple-negative; 32 ER-positive).

Her2 status was analyzed by immunohistochemistry (IHC) and reanalyzed by FISH if a score of 2+ was gained. ER was analyzed by IHC; ER negative tumours were defined by a cut-off value of <10% positively stained tumour cells. BMFS was estimated with the Kaplan-Meier product limit method and compared with the log-rank test; factors significantly associated with BMFS in the univariate analysis were included into a Cox proportional hazard model.

**RESULTS**

Median BMFS in triple-negative pts was 14 months (m) (95% CI 12.17-15.83), as compared to 25 m (95% CI 13.37-36.62) in Her2-positive (p=0.001) and 35 m (95% CI 19.79-50.22) in ER-positive pts (p<0.001), respectively. In Her2-positive pts, prior trastuzumab treatment for metastatic disease prolonged median BMFS (29 vs. 11 m; p=0.001); BMFS was further improved by trastuzumab in multiple lines (p=0.045) and co-positivity for ER and Her2 (30 vs. 15 m; p<0.001). ER-expression (HR 2.03; 95%CI 1.22-3.36; p<0.05) and prior trastuzumab (HR 2.72; 95%CI 1.20-6.17; p=0.017) remained independent predictors of longer BMFS in the Cox regression model. In ER-positive, triple-negative as well as Her2-positive pts, no correlation was found between BMFS and factors such as grading, histological subtype, stage IV disease at primary diagnosis, disease-free interval <24 months from primary treatment to diagnosis of metastatic disease, presence of visceral metastases, presence of lung metastases, and prior capcitabine exposure.

**CONCLUSIONS**

BMFS in triple-negative disease is significantly shorter as compared to Her2-positive or ER-positive tumours, mirroring the aggressiveness of this breast cancer subtype. Probably due to improved systemic disease control, trastuzumab significantly prolonged BMFS in Her2-positive pts. Longer BMFS in ER/Her2 co-positive disease reflects a less aggressive subtype of Her2-positive breast cancer which is less likely to benefit from strategies of BM screening or prevention.

**P4-17-06**

**Prognostic Role of Triple Negative Subtype in Breast Cancer Patients with Brain Metastases.**

Matthew A, Mathew IE, Rosenzweig MQ, Brufsky AM. University of Pittsburgh Medical Center; University of Pittsburgh Cancer Institute; Magee-Womens Hospital

**Background:** Metastatic breast cancer (MBC) patients with brain metastases (BM) have a poorer prognosis compared to patients with metastases to sites such as bone or other visceral organs. The role of breast cancer subtypes such as triple negative (TN) status and its relationship with other known prognostic factors have not been well delineated in the context of metastatic disease to the brain. We conducted a retrospective single institution cohort study of MBC patients with BM to evaluate the association between TN subtype and overall survival from the diagnosis of BM.

**Methods:** Baseline demographic and tumor specific data including ER, PR and HER2 status were collected on newly diagnosed MBC patients between January 1998 and December 2009. Overall survival was determined from the date of diagnosis of BM. Survival analyses were performed using the Kaplan-Meier method and Cox proportional-hazards model.

**Results:** Data were available on 186 MBC patients with BM, of whom 156 died during a median follow-up of 10.2 months from the diagnosis of BM; median age was 47.9 years. 91% of patients were Caucasian; 25.3% had triple negative disease. Median survival from the period of diagnosis of BM in patients with triple negative disease was 7 months (Interquartile range, IQR: 3-13) as compared to 11 months (IQR: 5-22) in patients who were HER2-positive or ER/PR-positive. Multivariate analysis found a higher risk of death after BM for TN disease subtype, with a hazard ratio for death of 2.89 (95% confidence interval: 1.89-4.44; p<0.001), when adjusted for variables such as age and stage at initial diagnosis of breast cancer, race, the number of metastatic sites, and the use of metastatic chemotherapy. The administration of metastatic chemotherapy had a significant survival benefit in the analyses, with a hazard ratio for death of 0.52 (95% CI: 0.27-0.99; p=0.048).

**Conclusion:** This retrospective cohort study in MBC patients with BM provides evidence for a greater risk of death in those with TN disease as compared to HER2-positive or ER/PR-positive subtypes even after adjusting for other prognostic factors.

**P4-17-07**

**The Shifting Landscape of Metastatic Breast Cancer to the CNS.**

Quigley MR, Fukai O, Chew B, Bhatia S, Karlovits S. Allegheny General Hospital, Pittsburgh, PA

**Introduction:** Improved survival following the diagnosis of breast cancer, in conjunction with new therapies and diagnostics has potentially altered the characteristics and course of patients presenting with CNS involvement.

**Methods:** Review of clinical and radiographic records of women presenting to a tertiary medical center with the new diagnosis of CNS metastatic disease from breast cancer. This was a retrospective review from patients identities obtained from two prospective databases. Results: There were 88 women analyzed who were treated over the period 1/2003-2/2010, average age 56.9. At the time of initial presentation of CNS disease, 68% of patients had multiple brain metastases, 17% had a solitary metastasis and 15% had only leptomeningeal disease (LMD). The median survival for all patients from the time of diagnosis of breast disease was 50.0 months, and 9.7 months from diagnosis of CNS involvement. The only factor related to overall survival was estrogen receptor positive (ER+) pathology (57.6 vs. 38.2 months, p=0.02 log-rank); those related to survival post CNS diagnosis were presentation with LMD (p=0.004, HR=3.1, Cox regression) and triple negative hormonal/HER2 status (p=0.02, HR=2.3, Cox regression). Patients with either had a median survival of 3.1 months (no patients in common). Of the 75 patients who initially presented with metastatic brain lesions, 20 (26%) subsequently developed LMD in the course of their disease (median 10.4 months), following which survival was grim (1.8 months median). Of note was that HER2+ status was protective in regard to late development of LMD (14.7 vs. 42.3%, p=0.03, Chi-square). Symptoms of LMD were most commonly lower extremity weakness (14/33), followed by cranial nerve deficits (11/33).
Conclusion: Treatment of patients with metastatic brain disease from breast cancer should be tailored to the patient’s hormonal status (as it markedly effects survival) and practitioners must be vigilant for the development of LMD, especially as it often presents with non-descript complaints such as back pain.

**P4-17-08**

Is Cranial Magnetic Resonance Imaging (MRI) Necessary for Staging of Asymptomatic HER2-Positive Breast Cancer Patients?

**Kaplan MA, Inal A, Kucukoner M, Urakci Z, Isikdogan A.** Dicle University, Diyarbakir, Turkey

**Background:** Breast cancer one of the most common tumors of the adults. Approximately 20% to 25% of all breast cancers are HER2-positive. It can be assumed that up to 30% of HER2-positive metastatic breast cancer (MBC) patients may experience brain metastasis during the course of their disease. The aim of this study is to investigate whether cranial MRI is necessary for staging in asymptomatic HER2-positive breast cancer patients.

**Material and methods:** HER2-positive breast cancer patients without symptoms of brain metastasis included in the study. Cranial MRI was added to the staging procedures. Subgroup analysis was performed to determine risk factors for developing brain metastasis.

**Results:** Seventy-five patients included in the study. Of whom, 71 were women (94.7%). Median age was 42.5 (22-76). Ten of the 75 patients (13.3%) had brain metastasis. The distribution rate of brain metastasis according to breast cancer stage at diagnosis was: 0% for stage I, 9.1% for stage 2, 4.3% for stage 3 and 28.6% for stage 4 (p = 0.027). There was no association between presence of brain metastasis and the site of metastasis, estrogen and progesterone receptor positivity, grade, histological subgroup, menopausal status and gender.

**Discussion:** Brain metastasis is an important problem for breast cancer patients. Treatment strategies may vary in the presence of brain metastasis. In our study we found brain metastasis nearly one quarter of patients with metastatic HER2-positive breast cancer. Cranial MRI imaging can be performed routinely in patient with metastatic HER2-positive breast cancer patients.

**P4-17-09**

Comparative Performances of Prognostic Indexes for Breast Cancer Patients Presenting with Brain Metastases.

**Braccinti A-L, Azria D, Thecasus S, Ferrero J-M, Romieu G, Jacot W. CRLC Val d’Aurelle Paul Lamarque, Montpellier; France; Antoine Lacassagne Cancer Center, Nice, France**

**Introduction:** Incidence of brain metastases (BM) from breast cancer has increased over the past decade, especially for HER2(+) tumours. Several scores and prognosis systems have been developed in the brain metastases (BM) setting in order to help physicians tailor treatment options depending on patient prognosis and to stratify patients enrolled in clinical studies. The aim of our study was to compare the clinical relevance of the major classifications and existing prognostic scores in a population of breast cancer patients affected by BM.

**Methods and Materials:** In this retrospective study conducted in Montpellier and Nice Cancer Centres, we retrospectively reviewed the clinical and biological data of 250 patients diagnosed with breast cancer BM between 1995 and 2010. Prognostic value and accuracy of recursive partitioning analysis (RPA), graded prognostic assessment (GPA), basic score for brain metastases (BS-BM) and a clinico-biological score (BS) developed in phase I study and validated in the BM setting were compared.

**Results:** Median age at BM diagnosis was 55 years-old. Most patients (74%) had good performance status (0-1). Brain was the first metastatic site in one third of patients (33.6%). In 12.4% of cases, no extracerebral metastases were detected at BM diagnosis. Among the 250 patients’ tumors, 43.6% overexpressed HER2 receptor and 25.6% were triple negative (negative estrogen receptor, progesterone receptor and HER2 status). After a median follow up of 4.5 years, median overall survival from BM diagnosis was 8.9 months (CI 95%, 6.9-10.3 months). The four scores were able to discriminate patients according to their survival. Chi2 tests showed a correlation between the different scoring systems. In multivariate analysis, the elimination model identified RPA (p = 0.031; hazard ratio, 0.77; 95% confidence interval, 0.64-0.91) and BS (p = 0.043; hazard ratio, 0.5; 95% confidence interval, 0.31-0.80) as the only two independent predictors of survival. RPA was the most accurate score in order to identify patients with high life expectancy, while RPA and BS were the most accurate scores to classify patients with short life expectancy.

**Conclusions:** In a general unslected population of breast cancer affected by BM, RPA seems to be the most useful score. Integration of biological parameters in addition of existing clinico-radiological scores is promising in order to improve the prognostic determination accuracy.

**P4-17-10**

Insights into Mechanisms Involved in the Accumulation of Nanovectors within the Breast Cancer Brain Metastasis.

**Yokoi K, Xuewu L, Alexander JF, Fidler IJ, Mauro F, Biana G. The Methodist Hospital Research Institute, Houston, TX; M D Anderson Cancer Center, Houston, TX**

Systemic chemotherapy for brain metastasis of breast cancer (BMBC) is considered to be inefficient. It is widely accepted in the literature that blood-brain-barrier (BBB) is responsible for clinical failures with the conventional chemotherapy. We hypothesized that nanovectors, therapeutic carriers with submicron dimensions, can be delivered to the brain tissue through a different pathway than molecular therapeutics. The objective of this study was to examine the accumulation of various intravenously injected nanovectors into the brain metastasis of breast and other cancers loci and to explore possible mechanisms involved.

Both human (MDAMB231) and mouse (4T1) breast cancer brain metastasis were evaluated. We also tested lung (3LL) and colon (CT26) originated cancers. Orthotopic brain tumors were produced either by intracarotid injection or stereotactic brain injection of cancer cells. When the animals became moribund, they were injected intravenously with one of the following nanovectors including fluorescently labeled PEGylated (100nm) and non-PEGylated (100nm, 250nm, 500nm) liposomes, disc-shape silicon particles (600, 1000nm in diameter). The accumulation of the nanovectors was compared to an unencapsulated fluorescent molecule. The accumulation of the nanovectors in the brain tumor tissue was evaluated through live animal imaging (IVIS) and immunohistochemical analysis. Immunohistochemical staining of endothelial cells and basal membrane markers was performed to determine the integrity of the tumor associated vasculature and the co-localization of the fluorescent nanovectors/molecules with tumor associated vasculature.

Interestingly, we have found that brain metastasis of different tumors (originated form breast, lung and colon cancers) possess dissimilar accumulation profiles for intravenously administered nanovectors. For example when comparing various tumor origins, 4T1 breast cancer originated brain tumors enabled the highest accumulation of the nanovectors as compared to CT26 and 3LL. On the other hand,
free molecule accumulated into the 4T1 brain metastasis model to a much lower extend than in nanovector. Finally, the observed brain distribution of nanovecortors corresponded well with impairment in vascular endothelium and basal membrane integrity shown in immunohistochemical analysis.

The results of our study point toward a possibility to treat specific patients with impaired BBB function with nanovector encapsulated chemotherapeutics based on the fact that their transport, accumulation and retention in the brain tissue are governed by different biological mechanisms, such as Enhanced Permeation and Retention (EPR) effect. These studies indicate that it is possible to cross the blood brain barrier, thus giving hope to patients with brain metastasis.

P4-17-11
Central Nervous System Involvement and Clinical Outcome, Review of 135 Patients, 9-Year Follow Up.
Watanabe J, Ogiya A, Tadokoro Y, Tanaka K, Takahashi K, Uematsu T, Mitsuya K. Shizuoka Cancer Center, Shizuoka, Japan; Shizuoka Cancer Center

Background: Breast cancer infiltrates into central nervous system (CNS) with the incidence of 10-20%. It is commonly accepted CNS screening does not contribute to maintain patients’ survival in metastatic breast cancer (MBC). We review our MBC cases with CNS involvement with the aim of searching prognostic factors and evaluating benefits derived from early detection of CNS lesion. Patients and Methods: From October 2002 to January 2011, 473 MBC patients treated at our hospital were reviewed and assessed. CNS lesion was found in 133 of 473 (28.5%) mostly by magnetic resonance imaging. Receptor statuses in patients with CNS disease were as follows; Estrogen receptor-positive 52.4%, HER2-positive 41.1% and triple-negative 12.7%. Asymptomatic patient at the diagnosis of CNS metastasis, in other words, CNS screening performed case, was 57.8%. Liver metastasis prior to CNS involvement was observed in 51.2% of patients.

Results: Within our 9-year follow-up, 122 patients received irradiation or operation, and 118 died. While major cause of death was lung or liver failure, 23.7% was considered as CNS death. Median overall survival from the diagnosis of primary breast cancer, survival from the MBC development and survival from CNS involvement were 1951, 1027 and 321 days respectively. Patients having HER2-disease showed superior survival after detection of CNS disease than HER2-negative patients. (median 514.0 vs 220.0 days, p=0.001) Asymptomatic patients, excluding underwent best supportive care (BSC), could not receive any survival benefit from early detection of CNS lesion. Liver metastasis prior to CNS involvement did not affect patients’ survival. Nineteen patients (14.1%), 17 of them were asymptomatic, received stereotactic irradiation as an initial treatment for CNS lesion, and showed superior survival after CNS event than patients received whole brain irradiation, surgery or BSC (median 670.0 vs 287.0 days, p=0.01).

Conclusion: Our review disclosed superior survival of HER2-disease with CNS metastasis. Early detection of CNS metastasis is only beneficial to patients when stereotactic irradiation will be suited for.

P4-17-12
Analysis of Predictive and Prognostic Factors for Metastatic Breast Cancer Patients with Brain Metastasis Treated by Whole Brain Radiotherapy.
Park IH, Lee KS, Shin KH, Ro J. National Cancer Center, Goyang, Korea

Background
We addressed the progression free survival of brain metastasis (PFS_BM) in metastatic breast cancer (MBC) patients treated by whole brain radiotherapy (WBRT) for brain metastasis (BM). In addition, we investigated predictive and prognostic factors for the PFS_BM and OS_BM.

Patients and methods
A total of 212 patients with BM, treated by WBRT at the National Cancer Center between January 2000 and December 2010 were reviewed. The PFS_BM was defined as the time interval from the date of start of WBRT to the date of a progression of metastatic lesions in the brain or death or last follow-up.

Results
The median age of patients was 45 years (range, 22-72 years) and the median time to brain metastasis was 12.7 months (range, 0.0-72.8 months).

Table 1. Patient characteristics (N=212)

<table>
<thead>
<tr>
<th>Variables</th>
<th>N=212</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (median, year, range)</td>
<td>45 (22-72)</td>
</tr>
<tr>
<td>HR (ER/PR)</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>50 (23.6%)</td>
</tr>
<tr>
<td>Negative</td>
<td>168 (79.4%)</td>
</tr>
<tr>
<td>Not known</td>
<td>14 (6.6%)</td>
</tr>
<tr>
<td>HER2</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>104 (49.1%)</td>
</tr>
<tr>
<td>Negative</td>
<td>101 (47.8%)</td>
</tr>
<tr>
<td>Not known</td>
<td>7 (3.3%)</td>
</tr>
<tr>
<td>Disease free interval (DFI) &lt; 2 yr</td>
<td>118 (55.7%)</td>
</tr>
<tr>
<td>ECOG PS</td>
<td></td>
</tr>
<tr>
<td>1-2</td>
<td>109 (60.2%)</td>
</tr>
<tr>
<td>≥3</td>
<td>103 (53.3%)</td>
</tr>
<tr>
<td>Not known</td>
<td>10 (4.8%)</td>
</tr>
<tr>
<td>No of metastatic lesions in brain</td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>224 (105.7%)</td>
</tr>
<tr>
<td>Oligo (&lt; 3)</td>
<td>17 (8.0%)</td>
</tr>
<tr>
<td>Multiple (≥3)</td>
<td>166 (78.7%)</td>
</tr>
<tr>
<td>Maximum LD of metastatic lesions in brain</td>
<td>22 (2-55)</td>
</tr>
<tr>
<td>Systemic disease status</td>
<td></td>
</tr>
<tr>
<td>At diagnosis</td>
<td>20 (9.4%)</td>
</tr>
<tr>
<td>CR</td>
<td></td>
</tr>
<tr>
<td>≥3</td>
<td>10 (4.8%)</td>
</tr>
<tr>
<td>PR</td>
<td>27 (12.7%)</td>
</tr>
<tr>
<td>SD</td>
<td>244 (116.0%)</td>
</tr>
<tr>
<td>PD</td>
<td>121 (57.1%)</td>
</tr>
<tr>
<td>Number of metastatic organs except brain</td>
<td></td>
</tr>
<tr>
<td>≥3</td>
<td>16 (7.5%)</td>
</tr>
<tr>
<td>≤2</td>
<td>66 (40.6%)</td>
</tr>
<tr>
<td>Not known</td>
<td>110 (51.9%)</td>
</tr>
<tr>
<td>Visceral involvement</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>165 (78.6%)</td>
</tr>
<tr>
<td>No</td>
<td>47 (21.4%)</td>
</tr>
<tr>
<td>Chemotherapy after WBRT</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>152 (71.7%)</td>
</tr>
<tr>
<td>No</td>
<td>50 (23.6%)</td>
</tr>
<tr>
<td>Not known</td>
<td>10 (4.8%)</td>
</tr>
<tr>
<td>LD-long diameter</td>
<td></td>
</tr>
</tbody>
</table>

LD-long diameter of all patients, 50 (23.6%) patients were hormone receptor (HR) positive and 104 (49.1%) HER2 positive. Six patients received a surgery or a focal radiotherapy for metastatic lesions prior to WBRT. Seventy one (33.4%) patients had well controlled extracranial systemic disease (CR+PR+SD) when brain metastasis was diagnosed. The median survival after BM (OS_BM) was 5.4 months (95% CI, 4.4-6.4 months) and approximately 14.6% of patients died mainly of progressive brain metastasis. The PFS_BM was significantly affected by poor performance status (PS ≥3) (P<0.001), uncontrolled systemic disease status (P=0.029), disease free interval (DFI) < 2 years (P=0.011), no chemotherapy after BM (P<0.001), and HER2 positivity (P=0.002). Of those, poor PS, no chemotherapy after BM, and uncontrolled systemic disease status were remained important factors on a multivariate analysis. In terms of OS_BM, poor PS...
(P<0.001), older age (P=0.005), number of systemic metastatic sites >3 (P<0.001), uncontrolled systemic disease status (P<0.001), disease free interval (DFI) < 2 years (P=0.014), no chemotherapy after BM (P<0.001), and visceral involvement (P=0.009) were significant factors. Of those, poor PS (P<0.001), uncontrolled systemic disease status (P<0.001), and no chemotherapy after BM (P=0.001) were important factors for shorter OS_BM when adjusted with other factors.

Conclusions
Uncontrolled extracranial systemic disease status, no chemotherapy after BM, and poor PS were significant factors for shorter PFS_BM and overall survival after brain metastasis.

P4-18-01
Prognostic Role of Tumor and Stromal p16/INK4A in Ductal Carcinoma In Situ (DCIS).

Linke SP, Bremer TM, Wärnberg F, Zhou W, Goldstein L, Jirström K, Amini R-M. Prelude Corporation, Laguna Hills, CA; Uppsala University Hospital, Uppsala, Sweden; PhenoPath Laboratories, Seattle, WA; Lund University, Lund, Sweden

Background: DCIS is a pre-invasive breast disease diagnosed in ∼50,000 women/year in the US. The standard treatment recommendation is for mastectomy or breast-conserving surgery plus radiotherapy (BCS+RT). However, only ∼10% of BCS patients avoid recurrence due to RT, and RT provides little or no survival benefit. Thus, it is important to stratify patients by risk to help tailor treatment to maximize benefit and minimize unnecessary adverse effects and costs.

Material and Methods: Tissue microarrays that included tumor cores from all women diagnosed with pure DCIS between 1986 and 2004 in Uppland and Västmanland, Sweden (n=458) were stained and scored for p16/INK4A (clone E6H4) using immunohistochemistry (IHC). Semi-quantitative manual scoring was done separately in the tumor epithelial cells and peri-tumoral carcinoma-associated fibroblasts (CAFs). Based on previous reports, thresholds to define low and high risk groups with 10-year recurrence rates of 38% and 7%, respectively, were censored if they had contralateral breast or other cancers as the first event.

Results: Patients were stratified into low and high risk groups using a multivariate model incorporating tumor p16, stromal p16, HER2, and margin status. Patients treated with BCS only (n=67) had a baseline 10-year recurrence rate of 31%. The model identified high and low risk groups with 10-year recurrence rates of 38% and 7%, respectively (hazard ratio=7.1, p=0.009). Similar results were achieved when BCS and BCS+RT patients were combined (n=130). Interestingly, all six recurrence events in the set of patients who received full mastectomies (n=43) were in the model high risk group.

Discussion: Previous studies have shown mixed results when p16 was used as a single marker. Our results suggest a prognostic interaction between tumor and stromal p16, as well as HER2, that will form the basis of a more comprehensive multi-marker IHC-based prognostic panel to assess risk of recurrence in DCIS patients.

P4-18-02
Sentinel Node Biopsy in Extensive Ductal Carcinoma In Situ (DCIS) Diagnosed by Vacuum-Assisted Macrobiopsy (VAMB) and Treated by Mastectomy: Results of the French Prospective Trial CINNAMOME.


Background: Lymph node evaluation in the management of DCIS has been completely abandoned as a result of the documented low incidence of nodal metastases (<2%) and the significant morbidity of lymph node dissection. However, the risk of occult invasive disease exists when the initial diagnosis is performed by vacuum-assisted micro biopsy (VAMB). Invasive disease is usually discovered during the histological analyses following mastectomy. Therefore, the only option for patients is complete axillary lymph node dissection (ALND). The aim of this study was to evaluate the number of ALND that can be avoided by using the sentinel lymph node (SLN) procedure to identify patients with invasive disease but no SLN involvement.

Trial design: Patients with extensive microlcalifications on mammography and DCIS diagnosed by VAMB treated by mastectomy were included in the study. The SLN procedure was performed and intraoperative evaluation on frozen sections was carried out. If the SLN was positive an ALND was performed during the same intervention. If the SLN procedure failed or was negative an ALND was not performed. Radiography of the mastectomy specimen was performed to assist the pathologist in confirming the DCIS diagnosis, to evaluate the size and to determine concordance rates between initial VAMB diagnosis and histological analyses. Results: Fourteen French cancer centers took part in this protocol over 2 years (May 2008-December 2010). 228 patients were enrolled, including 197 DCIS on VAMB. The SLN was identified in 193 cases (98%) but one case was not documented at histology leaving 192 valid cases for analysis.

Distribution of SLN results and histological lesions found on mastectomy specimens in the series

<table>
<thead>
<tr>
<th>Initial VAMB result Mastectomy result</th>
<th>Sentinel Lymph Node result</th>
<th>Treatment outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCIS 116</td>
<td>Positive</td>
<td>10 (ALND)</td>
</tr>
<tr>
<td>DCIS and micro invasive carcinoma 20</td>
<td>positive</td>
<td>4 (ALND)</td>
</tr>
<tr>
<td>DCIS and invasive carcinoma 56</td>
<td>negative</td>
<td>0 (ALND avoided)</td>
</tr>
<tr>
<td>DCIS and invasive carcinoma 56</td>
<td>positive</td>
<td>21 (ALND)</td>
</tr>
<tr>
<td>DCIS and invasive carcinoma 56</td>
<td>negative</td>
<td>35 (ALND avoided)</td>
</tr>
</tbody>
</table>

ALND was not performed for non-invasive disease and negative SLN (n=114) and invasive or micro-invasive disease and negative SLN (n=51). This meant that ALND was avoided for 67.1% of the patients with invasive disease (51/76, 95% CI[56.5-77.7]), or 26.6% of patients overall (95%CI [20.3-32.8]), whereas these patients would have previously received ALND without the use of the SLN procedure. We observed 39.6% (76/192) of discordance between VAMB results and definitive results from histology analysis after mastectomy across all patients.

Conclusions: SLN is a useful procedure for patients with DCIS diagnosed by VAMB treated by mastectomy and presenting extensive microlcalifications on mammography. For patients for whom microinvasive or invasive carcinoma is later identified on the mastectomy specimen, the use of this procedure makes it possible to spare over a quarter of them from ALND and the associated morbidity. Biological analyses are currently underway to determine predictive factors of invasive disease associated with DCIS.
P4-18-03
Basal-Like Phenotype of BRCA Associated Ductal Carcinoma In-Situ.
Kristjansdottir K, Laprise J, Scalia-Wilbur J, Simon R, Steinhoff M, Legare R. Women and Infants Hospital of RI, Providence, RI.

Background: Molecular profiling has identified different subtypes of invasive carcinoma. The basal-like tumors represent a subgroup that is known for aggressive behavior. The NCCN guidelines recommend genetic testing for patients with triple negative breast cancer as over 80% of breast cancers in BRCA1 mutation carriers are triple negative tumors, and 70% of those fulfill criteria for basal-like tumors. Ductal carcinoma in-situ (DCIS) is thought to represent a non-obligate precursor to invasive breast cancer. Little is known about basal-like precursor lesions and their association with germline BRCA mutations. The aims of this study were to characterize the immunohistochemical phenotype of BRCA associated DCIS and evaluate the clinical importance of such lesions.

Material and Methods: We identified 32 cases of DCIS from 28 women with a known BRCA1/2 mutation, from our genetics database. Tumor samples were reviewed to identify areas of DCIS for staining. Five micron sections were stained on the Dako Autostainer with antibodies to estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER-2), epidermal growth factor receptor (EGFR), and CK 5/6.

Results: 44% of the DCIS tumors were from BRCA1 mutation carriers and 56% from BRCA2 mutation carriers. 53% of the samples were triple negative (staining negative for ER, PR, HER-2) and 69% of the triple negative tumors were basal-like tumors (staining positive for either EGFR or CK5/6). The basal-like DCIS specimens were all high grade, and they all came from BRCA1 positive patients. 71% of BRCA1 positive DCIS had basal-like features.

Conclusions: DCIS in BRCA1 mutation carriers shows basal-like features that are in line with historical control for invasive carcinomas in the same group, suggesting that DCIS is a precursor lesion for BRCA1 positive cancer. Although further investigation is needed, our data suggest that patients with basal-like DCIS should be offered genetic testing.

P4-18-04
Hypofractionated Radiotherapy for Breast Ductal Carcinoma In Situ.
Hathout L, Fortin B, Vulpe H, Hijal T, Bahig H, Dubé P, Yassa M. Hôpital Maisonneuve-Rosemont, Centre Affilié à l’Université de Montréal, Montreal, QC, Canada; McGill University Health Centre, The Montreal General Hospital, Montreal, QC, Canada.

Background: Conventional radiotherapy at a dose of 50 Gy in 25 fractions after breast conservative surgery is the standard treatment for ductal carcinoma in situ of the breast (DCIS). Hypofractionation is an interesting alternative for the treatment of breast cancer, providing a less intense treatment scheme for the patient. While the hypofractionated regimen of 42.5 Gy in 16 fractions has been shown to be equivalent to 50 Gy in 25 in infiltrating ductal carcinoma, few studies and no prospective study have reported results using hypofractionation in DCIS.

Materials and Methods: In this multicenter collaborative effort, we retrospectively reviewed the records of women with a diagnosis of DCIS at two Canadian institutions, treated with conservative surgery followed by hypofractionated radiotherapy (42.5 Gy in 16 fractions) to the whole breast. Tumor, patient and treatment factors were collected. Local control was evaluated using the Kaplan Meier method. Curves were compared using log rank.

Results: Between 2003 to 2008, 292 patients with DCIS underwent conservative surgery followed by hypofractionated radiation in 16 fractions for a total dose of 42.5 Gy. Treatment delivery was similar at both institutions. Mean age at diagnosis was 59 years, with 70% of women being postmenopausal. Nuclear grade was 1-2 in 65% of patients while 28% had grade 3 tumors. Complete surgical excision with negative margins was achieved in 92% of patients. Radiotherapy boost was given to 92 patients (31.5%) at the discretion of the radiation oncologist. After a mean follow up of 3.35 years (range 1-8 years), 10 patients (3.4%) had ipsilateral local relapse resulting in a disease-free survival (DFS) of 96% at 4 years. The histology at recurrence was DCIS for 8 patients; infiltrative disease for one patient and one patient had an unknown histology at recurrence. Age was statistically associated with local relapse (DFS 89% for patients under 50 years old vs 97% for those older than 50, p=0.027). Grade was also a significant prognostic factor (DFS 98% for grade 1-2 vs 91% for grade 3, p = 0.015). Finally, administration of boost did not have an impact on local control (DFS boost 98% vs 95% for no boost, p=0.44).

Discussion: Hypofractionated radiotherapy (42.5 Gy in 16 fractions) provides excellent local control for patients with DCIS undergoing breast conservative surgery. This regimen is more convenient to both patient and physician since it is less time consuming. Further work needs to be done to determine if this regimen is as effective as conventional radiotherapy in younger women (age less than 50) and those with grade 3 tumors.

P4-18-05
Ductal Carcinoma In Situ with Microinvasion: Clinical and Pathological Characteristics, Treatments and Outcomes.

Background: Ductal carcinoma in situ with microinvasion (DCSIM) is a rising rare entity. Because of that and its controversial pathological definition, there is a lack of clear recommendations for treatment. The purpose of this study was to describe the clinical and pathological characteristics, treatments and outcomes of our single institutional experience.

Patients and methods: Individual clinical and pathological data were collected from 63 women, diagnosed and treated for DCISM at the Claudius Regaud Cancer Hospital between January 2000 and April 2010. All available histological material (45 patients) was reviewed by an expert pathologist.

Results: The median age was 56 years (range 34-83). Seven patients (11.5%) had a personal history of DCIS, 27 patients (42%) had a familial history of breast cancer. Fourteen patients (22%) had a clinical sign at the diagnosis. Fifty one patients were mammographically detected (81%). All the patients underwent surgery, mastectomy for 17 patients (27.4%) and conservative surgery for 45 (72.6%). Secondary surgery of the breast was required for 21 patients (46.6%) after conservative surgery, enlarging surgery (N=13) or mastectomy (N=8). Surgical axillary lymph node evaluation was performed on 52 patients (82.5%), axillary dissection alone for 10 patients, sentinel node biopsy alone for 37 patients and the 2 methods for 5 patients. The median size of the DCIS was 16mm (6-90mm) with 37/60 (61.7%) grade III Van Nuys classification. The most histological subtype was comedo carcinoma (68%). Concerning the 45 reviewed biopsies, the size of the microinvasive component was ≤ 1mm for 38 lesions and between 1 and 2 mm for 7 lesions. Hormonal receptor status was positive for 29 (64.4%), 27 (64.3%) for estrogen receptors and 20 (44.4%) for progesterone receptors. HER 2 status was performed.
for 34 patients, among 12 (35%) of them were found overexpressed on the microinvasive component. Lymph node invasion was found among 2 of the 52 patients (3.8%) who underwent axillary lymph node evaluation. Radiation therapy was delivered to all the patients after conservative surgery (n=37) (50 Gy with a 10 Gy boost for 22 of them) and 2 after mastectomy (chest wall irradiation (50 Gy)). Adjunct hormonotherapy was delivered on 11 patients (18%). With a median follow-up of 36.4 months (95% CI=[27.7-44.16]), 62 patients are alive at the last follow-up and 58/63 free of disease (2 relapses and 3 second cancers). The 3 year disease free survival rate was 91.1 (95% CI=[78.1;96.6]). During follow-up, two local relapses occurred on patients treated by mastectomy. One of them had local invasive relapse at 43 months and she is still NED after 90 months. The second patient who had local invasive carcinoma with axillary node invasion at 32 months treated by conservative surgery, axillary dissection, radiotherapy and chemotheraphy, had metastasis at 51 months and passed away after 70 months. Conclusion: Mammographic screening programmes increase the rate of small diagnosed tumours, specially DCISM. Despite a priori good prognostic outcome, 2 axillary node involvements and 2 delayed relapses were observed. So, this kind of presentation deserves better evaluation of relapse risk factors to determine adapted adjuvant therapies.

P4-18-06 Relationship between Pathological Features, Her2 Protein Expression, and HER2 and CEP17 Copy Numbers in DCIS.

Lambein K, Van Bockstal M, Praet M, Denys H, Braens G, Nuiys A, Cocquyt V, Pauwels P, Van Den Broecke R, Libbrecht L. Ghent University Hospital, Ghent, Belgium; Ghent University, Ghent, Belgium

Background Previous studies in which HER2 status in ductal carcinoma in situ (DCIS) was evaluated yielded conflicting results. The reported prevalences of HER2 overexpression and amplification vary remarkably. These discrepancies might partly be due to differences in assessment methods, i.e. FISH or immunohistochemistry (IHC) and usage of tissue microarrays or whole mount slides. To further investigate this issue, we performed both FISH and IHC for HER2, evaluated HER2 and CEP17 copy numbers and correlated these data with histopathological findings.

Methods
61 DCIS cases were studied, of which 55 pure DCIS and 6 DCIS with micro-invasion. Pathological features were evaluated and included: architecture, nuclear atypia, necrosis, calcifications, stromal inflammation, stromal morphology, extent of lesion, margin width, Van Nuys Prognostic Index and Pinder classification. Her2 IHC and HER2 dual probe FISH analysis were performed and scored according to ASCO/CAP guidelines. HER2/CEP17 ratio and HER2 and CEP17 copy numbers were separately analysed. IHC for estrogen and progesterone receptor (ER and PR) was also performed. Whole mount slides were used for all analyses.

Results
15 cases (25%) were scored negative (1+), 10 cases (16%) equivocal (2+) and 36 cases (59%) positive (3+) using Her2 IHC. According to FISH analysis, 34 of 60 cases (57%) showed HER2 amplification; there was insufficient tissue for FISH analysis in one case. The amplification status of the DCIS lesions correlated with the IHC score (p<0.001). 30 of all 34 amplified cases were assigned a 3+ IHC score, and remarkably, all these cases showed HER2 clusters on FISH analysis (88%). Amplified lesions showed more frequently nuclear atypia grade 3 (p=0.0335), extensive comedonecrosis (p=0.002) and stromal inflammation (p=0.003). HER2 amplification correlated with the presence of micro-invasion (p=0.0218%). There was no correlation with hormone receptor status or other pathological variables. In the amplified group, high-grade nuclear atypia was associated with a higher mean HER2 copy number (p=0.0026) and HER2/CEP17 ratio (p=0.0356), while this was not the case in the non-amplified group. CEP17 copy numbers did not correlate with nuclear atypia.

Conclusions
The correlation between HER2 amplification and adverse pathological features, including micro-invasion and the association in amplified DCIS between HER2 copy number and high-grade nuclear atypia, underscore that HER2 is a driver of DCIS aggressiveness and possibly of recurrence in the form of non-invasive cancer. However, the prevalence of HER2 overexpression, amplification and cluster formation was much higher than in invasive carcinoma, suggesting that HER2 might play a less important role in transition from DCIS to frankly invasive cancer. Further studies should evaluate non-invasive and invasive recurrence of resected DCIS separately.

P4-19-01 Male Breast Cancer According to Tumor Subtype and Race: A California Cancer Registry (CCR)-Population Based Study.

Chavez-MacGregor M, Clarke CA, Lichtensztajn DY, Hortobagyi GN, Giordano SH. The University of Texas, MD Anderson Cancer Center; Cancer Prevention Institute of California

Background: Breast cancer in men is a rare disease and accounts for 0.6% of all breast cancer cases. Prior reports suggest that male breast cancer typically has a ductal histology and is more likely than cancers of the female breast to express estrogen and progesterone receptors (ER and PR). To our knowledge, there are no population-based estimates of the rates of Her-2 positive breast cancer or of the distribution of breast cancer subtypes in male patients. The categorization of breast cancers according to tumor subtype has important implications in the prognosis and in the management of breast cancer patients. We explored the tumor subtype distribution by race/ethnicity in the large, ethnically diverse population of California.

Methods: Retrospective study using the CCR. Participants included all male breast cancer patients diagnosed with invasive breast cancer between 2005-2009 with known ER, PR and Her2neu status. Among the patients with hormone receptor positive (HR+) tumors, survival analysis using Kaplan-Meier method was used. Differences in probability of survival between groups were compared using log-rank test.

Results: Six-hundred and seven patients were included. Median age at diagnosis was 68 years. Ductal histology was present in 84.7% (n=514) of the cases, 51.6% (n=313) of all the tumors were classified as grade II. Four hundred and ninety four (81.4%) patients had HR+ tumors, defined as ER+ and/or PR+ and Her2neu negative. Ninety (14.8%) had Her2neu positive, and 23 (3.8%) had triple receptor negative tumors (TN). The majority of patients were white (71%), 11% were Hispanic, 7.6% black and 10.4% were Asian/Pacific Islander/other. The breast cancer tumor subtype varied significantly across races/ethnicities (p<0.0001). Among non-Hispanic Whites, 82.6% had HR+, 14.6% had Her-2+, and 2.8% had TN breast cancer. For Blacks and Hispanics the distributions were 73.9% HR+, 17.4% Her-2+, 8.7% TN and 77.6%, 16.4%, 6.0% respectively. Among HR+ patients, blacks and Hispanics were less likely to have PR+ tumors compared to whites. The rate of ER+PR- was 6.3% among whites, 19.6% among blacks and 16.4% among Hispanics (p<0.001).
survival analysis among HR+ patients showed significant differences according to race (p=0.018) and stage (p=0.001), with Black patients and patients with higher stage disease having poorer outcomes. **Conclusions:** In this large cohort of male breast cancer patients, the distribution of tumor subtype was different than what has been reported in females. Among males, differences in tumor subtype exist among different racial/ethnic groups. Blacks were more likely to have triple receptor negative tumors and more likely to have ER+/PR- tumors than white patients. Among HR+ patients, blacks experience the worse survival.

**P4-19-02**

A Population-Based Study of Contemporary Systemic Therapy for Male Breast Cancer.


Purpose: To describe the contemporary utilization, effectiveness and tolerability of tamoxifen, aromatase inhibitors, and trastuzumab in the treatment of early and advanced male breast cancer at a population level in British Columbia.

Patients and Methods: Records of 158 consecutive referred cases of invasive male breast cancer diagnosed from January 2000 to March 2010 were analyzed in a population-based cancer program with respect to patient and tumour characteristics, usage of endocrine agents, toxicity and medication adherence, trastuzumab usage and outcome. This group was compared to a population-based random sample of 1000 contemporary female breast cancer patients for prognostic and demographic factors. Survival outcomes were also compared to a cohort of women, matched 2:1 on prognostic and treatment factors.

Results: Pathology was most commonly infiltrating ductal carcinoma (94.3%) and estrogen-receptor positive (98.7%). Males were older (median 69.5 years) compared to women (median 60 years). Stage distribution was I (25.3%), II (43.0%), III (24.7%) and IV (7.0%), reflecting more advanced presentation compared to female cases I (43.9%), II (37.9%), III (12.5%) and IV (5.3%). Tamoxifen was prescribed as adjuvant therapy to 109 patients, and as palliative therapy to 11. Fourteen (11.7%) patients discontinued tamoxifen due to toxicity. Aromatase inhibitors were prescribed as adjuvant therapy for 16 patients and as palliative therapy for 24; these were discontinued due to toxicity in 5 patients (12.5%). Five of 9 patients known to over-express Her2 were treated with adjuvant chemotherapy with trastuzumab. Overall survival, breast cancer-specific survival and progression-free survival at 5 years were 72.4%, 85.5% and 79.7%, respectively. There was no statistical difference in outcomes compared to the matched female cases.

Conclusions: In this large population-based study of contemporary systemic therapy for male breast cancer, outcomes appear to be similar to those of women with the same diagnosis. Side effect profiles, tolerance, adherence and outcomes after tamoxifen, aromatase inhibitors and trastuzumab appear to be similar in men compared to that described in the literature for women.

**P4-19-03**

Physical and Psychological Sequelae of Breast Cancer in Men.

Ruddy KJ, Giobbie-Hurder A, Giordano SH, Goldfarb S, Kereaklogou S, Winer EP, Partridge AH. Dana-Farber Cancer Institute, Boston, MA; M.D. Anderson Cancer Center, Houston, TX; Memorial Sloan Kettering Cancer Center, New York, NY

Introduction: Little is known about the physical and emotional health of men with breast cancer. Information is also lacking regarding whether their informational and supportive care needs are being met regarding fertility and genetic concerns.

Methods: In an online pilot study recruiting participants through three websites focused on male breast cancer, www.outoftheshadowofpink.com, www.malebreastcancer.org, and www.malebreastcancer.ca, we surveyed men with breast cancer about their attitudes, symptoms, experiences, and health perceptions. We measured anxiety and depression using the Hospitalized Anxiety and Depression Scale (HADS), health-related quality of life (QOL) using Functional Assessment of Cancer Therapy-Breast (FACT-B), and hormonal and sexual symptoms using the Expanded Prostate Cancer Index Composite (EPIC) Hormonal and Sexual Scales. We assessed select toxicities from therapy and history of genetic and fertility counseling.

Results: Forty-two men responded to this online survey at least in part.

<table>
<thead>
<tr>
<th>Respondent Characteristics, N=42</th>
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<tbody>
<tr>
<td>Median age in years (range)</td>
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<td>Median time since diagnosis in years (range)</td>
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<tr>
<td>Stage</td>
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<td>4</td>
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<tr>
<td>Unknown</td>
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<tr>
<td>White</td>
</tr>
<tr>
<td>Married or “living as married”</td>
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<tr>
<td>Employed</td>
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<tr>
<td>Financially comfortable (have “money for special things”)</td>
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<tr>
<td>Treatment</td>
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<tr>
<td>Received chemotherapy</td>
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<tr>
<td>On tamoxifen</td>
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<td>On aromatase inhibitor</td>
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Results:

<table>
<thead>
<tr>
<th>Genetic Counseling and Testing</th>
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<tbody>
<tr>
<td>Referred for genetic counseling or testing</td>
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<tr>
<td>Had genetic testing for hereditary cancer syndrome</td>
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<tr>
<td>If tested, found to have a hereditary cancer syndrome</td>
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<tr>
<td>Fertility Issues</td>
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<tr>
<td>No biological children at time of survey</td>
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<tr>
<td>Desired biological children at time of survey</td>
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<tr>
<td>Informed about fertility risks of treatment</td>
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<tr>
<td>Stored sperm prior to treatment</td>
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<tr>
<td>Select Symptoms</td>
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<tr>
<td>Persistent menopausal symptoms</td>
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<tr>
<td>Chest wall pain</td>
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<tr>
<td>Lymphedema</td>
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<tr>
<td>Heart problem attributed to breast cancer treatment</td>
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<tr>
<td>Distress and Quality of Life</td>
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</tr>
<tr>
<td>Anxious (HADS Anxiety Score &gt;10)</td>
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<tr>
<td>Depressed (HADS Depression Score &gt;10)</td>
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<tr>
<td>FACT-B Score (higher=better QOL); Mean (SD)</td>
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<tr>
<td>EPIC Hormonal Score (higher=fewer symptoms); Mean (SD)</td>
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<tr>
<td>EPIC Sexual Score (higher=better sexual function); Mean (SD)</td>
</tr>
<tr>
<td>Rated ability to perform sexually over past 4 weeks “very poor”</td>
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</tbody>
</table>

Conclusions: Men with breast cancer experience substantial symptoms in follow-up. Sexual functioning, in particular, may be impaired (in men without cancer, mean EPIC Sexual Score is 61.4 with SD 23.6). Hormonal symptoms are also prevalent (in men without cancer, mean EPIC Hormonal Score is 91.7 with SD 9.7). We plan to use data from this pilot study to inform a larger study and develop targeted interventions to improve sexual functioning and reduce symptoms in male breast cancer survivors.
P4-19-04
Evaluation of Histopathological Parameters in Male Breast Cancer Reveals Differences Compared with Female Breast Cancer.
Nilsson C, Johansson I, Ahlin C, Thorstenson S, Bergkvist L, Amin R-M, Holmgqvist M, Hedenfalk I, Fjällskog M-L. Central Hospital of Västerås, Västerås, Sweden; Uppsala University, Uppsala, Sweden; Lund University, Lund, Sweden; Orebro University Hospital, Orebro, Sweden; Linköping University Hospital, Linköping, Sweden; Uppsala, Sweden

Purpose: To characterize male breast cancer (MBC) by evaluating established histopathological parameters and their prognostic value.

Methods: 197 male patients with invasive breast cancer and available paraffin-embedded tumor tissue were retrospectively assessed. Patient files were reviewed for clinicopathological data. Tumors were re-evaluated for histologic grading on conventional sections. Immunohistochemical (IHC) stainings for estrogen receptor (ER), progesterone receptor (PgR), human epidermal growth factor receptor 2 (HER2), silver-enhanced in situ hybridization (SISH), Ki-67, CK5/6 and epidermal growth factor receptor (EGFR) were performed on tissue micro arrays. Data on vital status and cause of death were retrieved from the Cause of Death Registry. Cox proportional regression models were used for uni- and multivariate analyses.

Results: ER and PgR positivity was demonstrated in 93 and 78% of patients, respectively. Nottingham histologic grade III was seen in 41% and HER2 positivity in 11% of all patients. Defining molecular phenotypes using IHC markers revealed luminal (ER+ and/or PgR+, and HER2-) in 83%, luminal/HER2+ (ER+ and/or PgR+, and HER2+) in 11%, basal-like (ER-, PgR-, HER2-EGFR+ and/or CK5/6 +) in 1%, but no cases of HER2-like or unclassified. Node-positivity (HR 4.6; 95% CI 1.9-11.4), tumor size > 20 mm (HR 2.3; 95% CI 1.1-5.0) and ER-negativity (HR 4.6; 95% CI 1.1-14.2) were significant risk factors for breast cancer death. Grade, HER2 status, Ki-67, age did not demonstrate independent prognostic information. No difference in breast cancer deaths was demonstrated between luminal and luminal/HER2.

Conclusion: Male breast cancer tumors seem to be of grade III more often than female breast cancer, whereas HER2 expression appears equally common. In our study, the most important predictors for breast cancer death in male breast cancer were lymph nodes, tumor size and ER status. The most common molecular phenotype was luminal.

P4-19-06
Ghrelin Expression Is Associated with Favorable Outcome in Male Breast Cancer.
Grönberg M, Nilsson C, Johansson I, Hedenfalk I, Janson ET, Fjällskog M-L. Dept. of Medical Sciences, Section of Endocrine Oncology, Uppsala, Sweden; Dept. of Oncology, Radiology and Clinical Immunology, Uppsala, Sweden; Dept. of Oncology, Clinical Sciences, Lund, Sweden; Center for Clinical Research, Västerås, Sweden

Background: Expression of the peptide hormones ghrelin and obestatin has previously been demonstrated in normal breast as well as in breast cancer. Interestingly, ghrelin expression has been shown to be associated with longer survival in female patients suffering from invasive breast cancer. However, the clinical implications of the expression of these peptides in male breast cancer are unclear. The aim of this study was to further investigate the role and potential clinical value of these peptides in male breast cancer.

Methods: A tissue microarray containing male breast cancer specimens from 197 patients was immunostained with antibodies directed towards the peptides. The expression of the peptides was evaluated and correlated to previously known prognostic factors in breast cancer and to the outcome. Cox regression analysis was used to assess whether these markers may predict survival of male breast cancer patients.

Results: Immunoreactivity for ghrelin and obestatin was observed in 81% and 87% of the cases, respectively. No strong correlations were found between ghrelin or obestatin expression and other known prognostic factors. Only ghrelin expression was significantly correlated to breast cancer-specific survival (HR=0.37-0.39, p=0.01-0.03) in both uni- and multivariate analyses. Reproducibility between the two readers was good to very good for both stainings with kappa values of 0.65-0.89.

Conclusions: Male breast cancer patients with tumors expressing ghrelin had 3 times lower risk for breast cancer death than those lacking ghrelin expression. Ghrelin expression is easily assessable with good reproducibility using immunohistochemistry. Further studies are needed to establish the clinical significance of ghrelin as a biomarker in breast cancer.
P4-20-01
Multicentric Phase II PACS 09/Beverly1 Trial: First Efficacy and Safety Results of Neoadjuvant Chemotherapy Combined with Bevacizumab in HER2-Negative Patients with Non-Metastatic Inflammatory Breast Cancer.

Viens P, Petit T, Dalenc F, Pierga JY, Delozier T, Romieu G, Bonnetere J, Ferrero J-M, Kerbrat P, Mouret-Reynier M-A, Bachelot T, Soulie P, Lerebourg F, Eymard J-C, Dehloeb M, Lortholary A, Hardy-Bessard A-C, Boher J-M, Asselan B, Charafe-Jauffret E, Lemontier J, Martin A-L, Andre F, Institut Pauli Calmettes, Marseille, France; Centre Paul Strauss, Strasbourg, France; Institut Claudius Regaud, Toulouse, France; Institut Curie, Paris, France; Centre François Baclesse, Caen, France; Centre Val d’Aurelle, Montpellier, France; Centre Oscar Lambret, Lille, France; Centre Antoine Lacassagne, Nice, France; Centre Eugène Marquis, Rennes, France; Centre Jean Perrin, Clermont-Ferrand, France; Centre Leon Berard, Lyon, France; Centre Paul Papin, Angers, France; Institut Curie, Saint Cloud, France; Institut Jean Godinot, Reims, France; Centre Alexix Vautrin, Nancy, France; Centre Catherine de Sienne, Nantes, France; Clinique Armoricaine, Saint Brieuc, France; R&D Unicancer, Paris, France; Institut Gustave Roussy, Villejuiff, France

Background: Inflammatory breast cancer (IBC) is a relatively rare form of breast cancer. Because this disease exhibits angiogenic properties, bevacizumab (BEV) may improve the efficacy of neoadjuvant chemotherapy (CT) in IBC.

Methods: Pts with HER2-negative IBC (IHC 0 or 1+ or FISH/CISH-, T4d, any N) were included to receive 4 cycles of FEC100 + BEV 15 mg/kg, d1 q3w, followed by 4 cycles of Docetaxel 100 mg/m2 + BEV 15 mg/kg q3w. Complete surgery was performed 4–6 weeks after the last dose of CT, followed by radiotherapy and endocrine therapy for receptor positive (HR+) pts. BEV was re-introduced as adjuvant therapy for 10 cycles, giving a total of 18 cycles of BEV. The primary endpoint of the PACS09/Beverly1 trial was to evaluate the pathologic complete response (pCR; Sataloff classification) rate in HER2-negative non-metastatic IBC patients.

Results: Between Jan 2009 and Sep 2010, 101 pts were included (100 pts were evaluable). Main baseline characteristics were: median age 49 years; postmenopausal status, 39 pts; SBR 2, 32 pts SBR3 61 pts, both HR-negative, 55 pts. 85 pts completed neoadjuvant therapy according to the protocol, 90 pts underwent complete mastectomy, 4 had a breast-conservative surgery. After neoadjuvant treatment, investigator-assessed pCR rate was 27% (18%-36%, CI 95%). Interestingly, a significantly greater proportion of pCR rate was observed in the HR-negative group compared to HR-positive group (38 vs 13% respectively; p=0.007). Results from central review of pCR are currently in progress. Main Grade 3,4 toxicities were: neutropenia (61 pts), febrile neutropenia (36 pts), mucitis (23 pts) Neither gr3/4 HTA nor cardiac failure or proteinuria was reported. There were no treatment-related deaths.

Conclusions: These data suggest that the use of BEV with neoadjuvant CT is active in HER2-negative IBC (pCR rate 27%), with an acceptable safety profile. Interestingly, the HR-negative subgroup is significantly more responsive than the HR-positive subgroup.

P4-20-02
Inflammatory Breast Cancer (IBC) in the National Comprehensive Cancer Network (NCCN): The Disease, the Recurrence Pattern and the Outcome.

Lubbe W, Li T, Hughes M, Ottesen R, Cristofanilli M, Weeks J, Wong Y-N, Fox Chase Cancer Center, Philadelphia, PA; Dana-Farber Cancer Institute, Boston, MA; City of Hope Cancer Center, Duarte, CA

Background: Inflammatory breast cancer (IBC) is a unique clinicopathologic entity that is characterized by rapid progression and aggressive behavior from the onset. The clinical presentation consists of erythema, rapid enlargement of the breast, skin ridging, and a characteristic peau d’orange appearance of the skin secondary to dermal lymphatic tumor involvement. Because of its uncommon presentation leading to frequent misdiagnosis, most reports are from small single institution series which describe a predictable pattern of recurrence in spite of appropriate multidisciplinary treatments. We sought to confirm these observations using the large multi-institutional National Comprehensive Cancer Network (NCCN) outcomes database.

Methods: Patients (pts) with newly diagnosed IBC treated between 1999 and 2009 at 12 participating NCCN institutions were identified. The clinical diagnoses of IBC was based on the AJCC definition and staged as clinical T4d, N0-3, M0-1. The baseline pathological characteristics included histological type, estrogen receptor (ER), progesterone-receptor (PR), and HER-2/neu status. Pts were classified as receiving multimodality therapy if they received two of the following three treatments: surgery (lumpectomy or mastectomy), perioperative (neoadjuvant or adjuvant) systemic therapy, or perioperative radiation therapy.

Results: We identified a cohort of 673 pts with newly diagnosed IBC with a median follow-up of 28.9 months. Of which 195 (29%) had metastatic disease at presentation. The median age at presentation was 52.6 years. Caucasians were 79.4% of the cohort, African American 9.7%, and 11.0% other ethnic groups. Invasive ductal type comprised 84% of histologies. Biomarker assessment revealed ER+ (44.7%), PR+ (34.3%), and Her2/neu+ (33.4%). LVI was documented in 53.3%. Of stage III patients, 75.7% pts received perioperative radiation, 82% received perioperative systemic therapy and 70.7% underwent surgery. All three modalities were received by 64.4% of women. Of the stage III pts, 203 recurred. The most frequent sites of recurrence for were CNS (20.2%), bone (17.2%), chest wall (13.8%), lung (12.3%), liver (11.3%), distant (7.4%) and regional lymph nodes (6.9%). With a median of 30 and 20 months of follow-up for stage III & IV respectively, the median survival was 66 months (95% CI 54-107) for stage III pts and 26 months (95% CI 22-33) for stage IV. Among the 82% of stage III pts who received multimodality therapy, the 5 year and 10 year OS of 62% and 47%.

Discussion: This is a large retrospective multiinstitutional study that confirms the aggressive clinical features, recurrence patterns and adverse prognosis of IBC described in previous single institution series. Even with aggressive multimodal therapy, the long term survival of IBC shorter is than non-IBC. Future investigations are needed to address the aggressive biology of IBC to improve diagnosis and therapy.
P4-20-03

T-Cell Cytokine Production Related to Progression of Breast Cancer Patients.

Cohen EN, Gao H, Lee B-N, Giordano A, Tin S, Anfossi S, Parker CA, Cristofanilli M, Valero V, Alvarez RH, Hortobagyi GN, Woodward WA, Ueno NT, Reuben JM. The University of Texas MD Anderson Cancer Center, Houston, TX; Fox Chase Cancer Center, Philadelphia, PA; The University of Texas Graduate School of Biomedical Sciences at Houston, TX

**Background:** Impaired immunosurveillance and immune dysregulation contribute to the pathogenesis and progression of breast cancer (BC). Upon activation, T cells synthesize inflammatory cytokines such as TNF-α that can promote or inhibit tumor growth. We therefore investigated T-cell cytokine syntheses as a predictor of disease progression.

**Methods:** We recruited 115 BC patients [25 with locally advanced breast cancer (LABC), 21 with metastatic breast cancer (MBC), and 44 with metastatic IBC (mIBC)] and 31 healthy donors (HD) for this ongoing study. The tumor phenotype consisted of 69 hormone receptor (HR) positive (including 26 patients with HER2 positive disease), 16 HR negative but HER2+, 30 triple negative BC (TNBC). To evaluate T cell function, peripheral blood mononuclear cells from patients and HD were stimulated overnight with immobilized anti-CD3 and soluble anti-CD28 antibodies and assessed for the percentage of T cells that synthesized cytokines by multi-parameter flow cytometry. The associations of T cell cytokine production profile with patient progression free survival (PFS) were analyzed by Kaplan Meier Test.

**Results:** The median follow-up (FU) of 113 evaluable patients was 14.1 months with a median time to relapse of 10.5 months; 54 patients had stable disease (SD) and 59 patients had progression of disease (PD). In the entire cohort, on univariate analysis, metastasis, BC, stage, and previous treatment predicted for worse PFS (p<0.05). In non-metastatic patients (LABC+IBC), absolute count of anti-CD3 activated CD8+ T cells producing IL-17 was significantly higher in the SD patients compared with patient with PD (p=0.038), but it did not predict PFS (p=0.073). Similarly in metastatic patients, anti-CD3 activated CD4+ T cells producing TNF-α were significantly higher in patients with SD (p=0.025) and were predictive of longer PFS (p=0.033). Considering all patients with IBC (IBC + mIBC), although patients with PD had significantly fewer (percent and absolute number) anti-CD3 activated T cells capable of producing cytokines, this immune impairment was mostly related to metastasis and previous treatment. However, the percentage of anti-CD3 activated CD8+ T cells producing TNF-α was an independent positive prognostic indicator of PFS (p=0.002).

**Conclusion:** Higher than average cytokine syntheses by anti-CD3 activated T cells are significantly associated with longer PFS. These data are consistent with the hypothesis that an adaptive immune response can control disease progression.

P4-20-04

Cytokine Synthesis by Activated Dendritic Cells in Relation to Disease Progression in Inflammatory Breast Cancer (IBC).

Gao H, Cohen EN, Lee B-N, Giordano A, Tin S, Anfossi S, Parker CA, Cristofanilli M, Valero V, Alvarez RH, Hortobagyi GN, Woodward WA, Ueno NT, Reuben JM. The University of Texas MD Anderson Cancer Center, Houston, TX; Fox Chase Cancer Center, Philadelphia, PA

**Background:** Deficiencies in innate and adaptive immune responses by plasmacytoid dendritic cells (pDC) and myeloid DC (mDC) have been linked to poor clinical outcome in breast cancer (BC) (Treilleux, Clin Cancer Res, 2004, PMID 15569976). pDC produce IFN-α and pro-inflammatory cytokines that regulate innate and adaptive immunity in breast cancer. mDC present in blood and secondary lymphoid organs secrete IL-12 and induce inflammatory cytokine production by T cells. Therefore, we studied DC activity in the peripheral blood and assessed their function with clinical outcome in breast cancer patients.

**Methods:** We recruited 115 BC patients [25 with locally advanced non-IBC (LABC), 25 with IBC, 21 with metastatic breast cancer (MBC), and 44 with metastatic IBC (mIBC)] and 31 healthy donors (HD) for this study. Peripheral blood pDC and mDC were activated through toll-like receptor (TLR)-7 to assess IFN-α and IL-10 production whereas mDC were activated through TLR-8 to assess production of IL-12 and TNF-α by multi-parameter flow cytometry. Associations between cytokine production by TLR-activated pDC and mDC with progression free survival (PFS) and overall survival (OS) of patients were analyzed by Kaplan Meier Test.

**Results:** The median follow-up (FU) of 113 evaluable patients was 14.1 months with a median time to progression of 10.5 months; 54 patients had stable disease (SD) and 59 had progression of disease (PD). Metastasis, previous treatments, and IBC contributed to shorter PFS and OS. Compared to HD, BC patients had significantly fewer total DC (p=0.008), mDC (p=0.008), and pDC (p=0.003) per µL. In general, the number of TLR-7-activated pDC per µL that produced IFN-α (p=0.023) or IL-10 (p=0.027) and the number of TLR-8-activated mDC per µL that produced IL-12 (p=0.001) and TNF-α (p=0.008) were significantly lower in BC patients than in HD. However, patients with DC that produced these cytokines above the median levels of HD had shorter PFS or OS. In BC patients, higher numbers of TLR-8-activated mDC that produced TNF-α (p=0.025) or IL-12 (p=0.003) predicted shorter OS. In mIBC patients, a higher number of TLR-7-activated pDC producing IFN-α (p=0.024) or IL-10 (p=0.034) predicted shorter PFS.

**Conclusion:** BC patients had significantly fewer pDC and mDC in peripheral blood than HD. BC patients with above average numbers of TLR-activated DC capable of producing proinflammatory cytokines had a significantly shorter PFS or OS. Disease progression in IBC is related to an increased number of activated dendritic cells producing inflammatory cytokines.

P4-20-05

Inflammatory Breast Cancer: Comparison of Epidemiology, Biology, and Prognosis between Japan and the United States, a Hospital-Based Study.

Yamauchi H, Natori A, Hayashi N, Soejima K, Takahashi O, Fukui T, Nakamura S, Cristofanilli M, Ueno N. St. Luke's International Hospital, Chuo-ku, Tokyo, Japan; Showa University, Shinagawa-ku, Tokyo, Japan; Fox Chase Cancer Center, Philadelphia, PA; The University of Texas M.D. Anderson Cancer Center, Houston, TX

**Background:** Inflammatory breast cancer (IBC) is a challenging disease characterized by low incidence, rapid progression, and poor survival. The epidemiology of IBC has been slow to emerge, given that the rarity of IBC makes large prospective clinical trials difficult. Compared to non-IBC, well-established features of IBC include a younger age of onset and lower frequency of hormone receptor positivity. Higher body mass index (BMI) is a purported risk factor. Previous reports also suggest that IBC is associated with more racial disparities, including incidence and age at diagnosis. The epidemiology of IBC in East Asia, however, has not been investigated. We performed a comparative study of IBC in Japan...
versus the United States to determine its epidemiologic and clinical features and to evaluate the differences in epidemiologic factors between the two countries.

Patients and Method: Patients who visited St. Luke’s International hospital (SLIH) in Tokyo, Japan and The University of Texas MD Anderson Cancer Center (MDA) in Texas, USA between 2003 and 2009 were identified. Epidemiological and biological data were collected from electronic medical records. Patient and tumor characteristics were tabulated and stratified by hospital. Kaplan-Meier curves were created for survival estimates and log-rank test was used for cross-group comparisons. Cox proportional-hazard analysis was used to identify a subset of significant prognostic variables that related to overall survival.

Result: 34 patients at SLIH and 531 patients at MDA were identified. Mean age at diagnosis was 52 years old (range, 32-81, SD, 10.8) and 50 years old (range, 22-87, SD, 11.6), respectively (P=0.476). Mean BMI was 22.9 kg/m² (range, 17.3-30.5, SD 3.3 ) and 31.0 kg/m² (range, 13.6-88.9, SD, 7.8) respectively (P<0.01). Clinical Staging was not significantly different; Stage IIIB 38.2%, Stage IIIC 26.5%, and Stage IV 32.4% at SLIH versus 48.6%, 23.7%, and 27.3% at MDA (P= 0.167). Estrogen receptor (ER) and progesterone receptor (PR) negative cases were, respectively, 50.0% and 64.7% at SLIH and 50.5% and 64.2% at MDA (ER, P= 0.935; PR, P=0.908). Her-2 over-expression cases were 38.2% at SLIH and 28.6% at MDA (P=0.174). A significant difference in nuclear grade was seen between SLIH and MDA: 20.6% at SLIH were Grade 3 versus 68.7% at MDA (P<0.01). Median overall survival at SLIH was 3.6 years versus 2.3 years at MDA (P=0.570). No prognostic factors were associated with overall survival.

Conclusion: Though IBC at SLIH differed significantly from IBC at MDA by several epidemiologic and biologic factors, there was no significant difference in survival. To define the epidemiological, prognostic, and risk factors of IBC in Japan, as well as in the world, further studies are needed.

P5-01-01
Identification, Validation and Assessment of Transcriptional Relevance of a PDGFR-Activation Signature in (Inflammatory) Breast Cancer.
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Introduction. Breast cancer can be divided into several subgroups characterized by unique patterns of pathway activation. Platelet-derived growth factor receptor (PDGFR) signalling has not yet been included in this classification scheme, although it has been reported to be a potential target for therapy. In this study, we have constructed a PDGFR-activation signature and investigated its relevance in breast cancer.

Materials and Methods. Sixteen PDGFR-modulated genes were identified by intersecting two published PDGFR-modulated gene lists. The resulting gene signature was applied onto a publicly available gene expression data set of GIST (GSE17743) using principle component analysis. The segregation of PDGFR- and KIT-mutated GIST samples was investigated using permutation analysis and classification sensitivity and specificity were assessed. Using the regression coefficients from the first principal component, a PDGFR-activation score was constructed and applied onto a second data set in order to validate the score (GSE1923). Finally, the score was applied onto a gene expression data set of 389 breast cancer samples, including 137 samples from patients with IBC.

Results. Sixteen PDGFR-modulated genes (NR4A1, EGR3, JUNB, IER3, TIEG, JUN, BCL3, MYC, NR4A3, PLAU, MCL1, DUSP1, DUSP5, DUSP6, SGK, GADD45A) were able to discriminate PDGFR-mutated GIST samples from KIT-mutated GIST samples with a sensitivity of 75% and a specificity of 85%. Application of the PDGFR-activation score onto a data set of control and PDGF-treated glioblastoma cells showed a significant increase in the PDGFR-activation score in the treated condition (P=0.0302). Application of the PDGFR-signature onto our series of IBC and nIBC samples demonstrated a significant and molecular subtype-independent increase in PDGFR-activation in IBC (P=0.0015; FDR=3%). In addition, in our series of nIBC samples only, PDGFR-activation was associated with decreased DMFS and RFS (P<0.0038 and P=0.0137 respectively). In fact, PDGFR-activation was an independent prognosticator in a multivariate model incorporating the molecular subtypes.

Discussion. We identified a gene signature composed of 16 genes able to predict PDGFR-activation in tissue samples by gene expression analysis. PDGFR-activation is significantly increased in samples from patients with IBC, an aggressive form of locally advanced breast cancer. In addition, in nIBC, PDGFR-activation is associated with DMFS and RFS, independently of the molecular subtypes suggesting that PDGFR-activation might add another level of clinically relevant heterogeneity in breast cancer.

P5-01-02
Wnt5a Is a Prognostic Biomarker in Estrogen Receptor-Positive Premenopausal Breast Cancer.
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Background: Wnt proteins belong to a family of secreted growth factors, which are involved in a wide range of cellular processes. Wnt signaling can be broadly divided into two categories; the canonical, β-catenin-dependent pathway and the non-canonical β-catenin-independent pathway. Wnt5a is a non-canonical signaling member of the Wnt family. Studies in two smaller cohorts of breast cancer revealed that loss of Wnt5a was associated with early relapse, metastatic disease and poor survival. Moreover, reconstitution of Wnt5a signalling decreased the migratory capacity and invasiveness of cultured breast cancer cells and administration of a Wnt5a-mimicking peptide was proven to inhibit metastases in a mouse model.

Material and methods: Premenopausal women (n=564) were included in a controlled randomised trial and allocated to tamoxifen for two years versus no adjuvant treatment. A tumour tissue microarrays was constructed and 390/500 samples were evaluable for Wnt5a protein expression by immunohistochemistry. Only cases with at least 2 evaluable cores were included. Wnt5a levels were determined semiquantitatively (scores 0-3). Clinical and pathological data such as patient age, histological subtype, pT stage, tumor size, pN stage, ER, PR, Ki67 and HER2 status were available. The endpoint was 10 year recurrence-free survival.

Results: Wnt5a protein expression was lost in 158/390 of the analyzed tumors and more common in younger (<45 yrs) women (p=0.032). Furthermore, loss of Wnt5a was significantly associated with ER and PR negativity (p=0.019 and p= 0.033, respectively). Loss of
Wnt5a was significantly correlated to poor survival in the whole cohort, \( p=0.02 \). When analyzing the data according to breast cancer subtype, we found that Wnt5a was a strong independent prognostic factor in ER+ breast cancer \( p=0.009 \), but not in ER- breast cancer \( p=0.8 \). This was true both in the adjuvantly treated and untreated cohorts, verifying that Wnt5a is a true prognostic factor and not a predictor of response to therapy.

**Discussion**: While associated with a better short-time prognosis than ER negative tumors, a subset of ER positive breast cancer patients are known to experience tumor recurrences >5 years after primary treatment and hence have a dismal prognosis. Those patients need to be identified and their tumors targeted with more aggressive treatment regimens. We have identified and validated Wnt5a as a prognostic biomarker in ER positive breast cancer in premenopausal women. Wnt5a signalling could potentially be reconstituted by administration of a Wnt5a-mimicking peptide, shown to inhibit metastases in mice, thereby offering ER positive premenopausal patients a targeted adjuvant therapy that potentially could lower their risk of metastatic disease.

P5-01-03
**Correlation between Aromatase Expression in Metastatic and Primary Breast Cancer.**
Ribeiro JM, Luis IV, Correia I, Casimiro S, Fernandes A, Quintela A, Mestan J, Ramos M, Costa L. Hospital de Santa Maria, Lisbon, Portugal; University of Lisbon, Lisbon; Hospital de Santa Maria, Lisbon; Novartis Pharma

Background: Estrogens are the major mitogenic factor in hormone dependent breast cancer. In postmenopausal women, estrogen production results mainly from extra-glandular conversion of androstenedione to estrone. This conversion is catalyzed by the enzyme aromatase (Aro). It is known that an higher expression and activity of the enzyme Aro is present in primary breast carcinoma compared with normal breast tissue. These data may indicate that tumors have the ability to produce their own estrogen. However, it is not known whether this process can occur at the level of metastasis. This study aims to determine the expression of aromatase in metastatic breast cancer and to analyze its correlation with expression at primary tumor and response to aromatase inhibitors (AI).

Material and Methods: We selected 41 patients with metastatic breast cancer, followed in the Department of Medical Oncology since 1995, undergoing surgery or biopsy of a metastatic lesion in the course of the disease (24 liver metastases; 10 bone metastases; 6 lung metastases; 1 lymph node metastases). For all these patients a detailed clinical chart review regarding clinical information (including treatment and tumor status) was obtained. Samples (formalin fixed and paraffin embedded) were analyzed for aromatase expression by immunohistochemistry (IHC), using the anti-aromatase monoclonal antibody # 677 (Novartis). Slides were scored considering the proportion of immunoreactive cells and intensity of the staining, and evaluating separately the epithelial and stromal component. The Fisher and log-rank test were used to determine the correlation between Aro expression in metastatic tissue and response to AI.

Results: Aromatase was expressed in 51% (21/41) of the samples (13 liver metastases; 8 bone metastases). The average combined score was 130 (10 - 320). The average score was higher in the epithelial component versus the stromal component (123 vs. 17.5). There was a strong expression of Aro in bone metastases (average combined score: 176) compared with other sites. There was no correlation between Aro expression and estrogen receptors expression. In 15 patients, it was also possible to evaluate the expression of Aro in the primary tumor. Aro was expressed in four cases (36%), with an average combined score of 52.5. The Aro expression between the primary tumor and metastasis was concordant in nine cases.

Thirty-four patients received AI to treat metastatic disease. The median time to progression (TTP) with AI in the entire population was 17.8 months. In the group of patients with Aro expression in metastasis, the median TTP in AI was 19.5 months, significantly higher than that obtained in the group without detectable Aro expression: 6.5 months (\( p = 0.045 \)). The overall survival in the group with Aro expression was 125 months and the 77 months in patients without Aro expression (\( p = 0.54 \)).

Conclusions: Aromatase is expressed by metastatic tumor cells from breast cancer, with a positive and statistically significant correlation between its expression in the metastasis and the clinical benefit to IA. Its expression was higher in bone metastases compared with other sites of metastasis although this difference was not, in our study, statistically significant.
standard IHC also showed a similar trend (median H score 100, 150 and 120, respectively) with a significant increase in the normal vs. DCIS comparison (p=0.0090). FOX3 lymphocyte count had a positive correlation with FOX3 epithelial intensity in the normal and invasive components (Spearman ρ=0.4343, p=0.0130 for normal and Spearman ρ=0.5456, p=0.0012 for IDC). FOX3 lymphocyte fraction and FOX3 epithelial intensity did not vary significantly with size of invasive tumor, nodal status, or stage of disease.

Discussion: FOX3 is expressed both by tumor infiltrating T lymphocytes as well as tumor epithelium. The significant increase in FOX3 T regulatory lymphocytes and FOX3 epithelial expression from normal to invasive cancer suggests a role of FOX3 in breast carcinogenesis and progression. The significant correlation of FOX3-T regulatory lymphocytes and FOX3 epithelial expression in the normal and invasive components suggests expression of one may influence the other early in the breast cancer progression pathway.

P5-01-05
Activating Mutations in PIK3CA or AKT1 in the I-SPY 1 Trial (CALGB 150007/150012; ACRIN 6657).
Boudreau A, Yau C, Petrelli L, Stemke-Hale K, Mills GB, Gray JW, Wolf DM, van ’t Veer LJ. The I-SPY 1 TRIAL Investigators, Esserman LJ. University of California, San Francisco; University of Texas MD Anderson Cancer Center; Oregon Health & Science University

Background: Mutations in the catalytic domain of phosphatidylinositol 3-kinase (PIK3CA) are among the most frequently observed activating mutations in breast cancer. We used the I SPY 1 TRIAL, a group of biologically and clinically high risk patients molecularly profiled and treated with neoadjuvant chemotherapy, to determine the frequency of mutations and their relationship to pathologic complete response (pCR) and outcomes, within the entire cohort and within subtypes defined by growth and hormone receptor (HR) expression.

Methods: Patients enrolled in the I-SPY 1 TRIAL had a tumor size ≥3.0cm and were administered a doxorubicin-containing regimen, followed by a taxane, prior to surgery. Sequenom single nucleotide polymorphism (SNP) profiling was performed on breast tumor genomic DNA isolated from a subset of patients (n=152). A total of 149 SNPs covering 16 genes (including PIK3CA and AKT1/2/3) were analyzed. Mutations were tested for association with estrogen receptor (ER), progesterone receptor (PgR), and HER2 status, as well as pCR, using Fisher’s exact test; associations between mutations and recurrence-free survival (RFS) were measured by log-rank tests. pCR was defined as no invasive tumor present in either the breast or axillary lymph nodes following neoadjuvant treatment.

Results: Of 149 mutations profiled in the cohort, 13 of the SNPs were observed. PIK3CA mutations were the most frequently observed in the panel (15.1%), followed by AKT1(E17K; 2.7%), CTNNB1(D32; 1.4%), NRAS(Q61; 0.7%), and FGFR2(N549; 0.7%). Mutations in PIK3CA or AKT1 were associated with ER-positivity (p=0.0047) and PgR-positivity (p=0.044). Within receptor subtypes, the frequencies of PIK3CA/AKT1 mutations were also significantly different (HR+HER2+: 27%; HR+HER2-: 14%); HR-HER2+: 0% (0/36), p<0.0008). Unlike previous reports (Loi et al, PNAS 2010), no significant association between PIK3CA/AKT1 mutation status and RFS was observed when we restricted our analysis to the adjuvant endocrine treated subset of the HR+HER2- patients (n=49; log rank p = 0.369). In contrast, and similar to cell line reports (Juntilla et al, Cancer Cell 2009), PIK3CA mutations appears to associate with worse RFS within the small subset of trastuzumab treated HER2+ patients (n=22, 13 HR-HER2+; 9 HR+HER2-; log rank p=0.001), suggesting mutations may influence response. Similar analyses of a larger cohort are planned to confirm these observations.

Conclusions: Within the I-SPY 1 TRIAL cohort, PIK3CA and AKT1 mutations are much more frequent in the HR+ and HER2 subsets but are not predictive of response to therapy or outcome except potentially within the HER2+ subset. The potential link observed between activating PIK3CA/AKT mutations and trastuzumab resistance merits further investigation, as it may provide a clinical rationale for testing PIK3CA mutation status in HER2+ patients and investigating combination therapies targeting this pathway, particularly in the HER2-+HR+ subset which have an elevated risk for recurrence despite pCR and trastuzumab therapy.
2) a trend towards worse outcome with higher TYMS expression in a) the overall population (TTP-HR 1.23, 95% CI 0.93 to 1.64, p=0.148), b) in ER+ patients (TTP-HR 1.46, 95% CI 0.97 to 2.19, p=0.07) as well as in c) HER2- patients (TTP-HR 1.17, 95% CI 0.85 to 1.61, p=0.343), 3) a statistically significant association between higher TYMP expression and longer PFS, but not TTP in ER+ and HER2- patients (table).

Discussion: Our ASCO 2011 analysis showed that high TYMS GCN is predictive of poor outcome in ER+ and HER2- patients, consistent with the fact it is the target of C. Here, we show that 1) expression of TYMS is significantly correlated with GCN 2) higher TYMS expression demonstrates the same trend towards poor outcome in ER+ and HER2- patients as in FISH, 3) higher TYMP expression is significantly associated with longer PFS in ER+ and HER2- patients, consistent with its C activating role. Differential sensitivity between FISH and DASL might be explained by the fact that DASL is performed in a pool of RNA coming from many cellular types, whereas FISH is scored selectively in tumor cells. These findings suggest that TYMS and TYMP GCN and expression can be useful predictive markers of C sensitivity in human breast cancer.

Identification of SORBS2 as a Candidate Marker To Predict Metastatic Relapse in Breast Cancer.

Background: Elucidation of promising cancer biomarkers from gene expression data can provide important insight into the relationship between signaling networks and cancer. SORBS2, sorbin and SH3 domain containing 2, is a multi-adapter protein involved in signal transduction associated to the cytoskeleton and was reported to be strongly repressed in pancreatic and cervical cancers.

Methods: With the purpose of identifying genes involved in metastatic process, we compared gene expression profiling of 19 invasive ovarian cancers and 24 borderline tumors. Prognostic value of the selected genes was then tested in a gene expression array database that includes 1659 patients with early breast cancer (Gyorffy B et al. 2010). Upon isolation of SORBS2 as a predictor, its involvement in cell migration and tumor progression was investigated in vitro. Small interfering RNA targeting SORBS2 was used to downregulate its expression in T47D and Hela, two cell lines overexpressing SORBS2. Functional effect of siRNA-induced knockdown of SORBS2 on cell viability was determined by WST-1 assay and Trypan Blue exclusion test. Effect of siRNA-induced knockdown of SORBS2 on cell viability was performed in a pool of RNA coming from many cellular types, whereas FISH is scored selectively in tumor cells. These findings suggest that TYMS and TYMP GCN and expression can be useful predictive markers of C sensitivity in human breast cancer.

**P5-01-07**
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Methods: With the purpose of identifying genes involved in metastatic process, we compared gene expression profiling of 19 invasive ovarian cancers and 24 borderline tumors. Prognostic value of the selected genes was then tested in a gene expression array database that includes 1659 patients with early breast cancer (Gyorffy B et al. 2010). Upon isolation of SORBS2 as a predictor, its involvement in cell migration and tumor progression was investigated in vitro. Small interfering RNA targeting SORBS2 was used to downregulate its expression in T47D and Hela, two cell lines overexpressing SORBS2. Functional effect of siRNA-induced knockdown of SORBS2 on cell viability was determined by WST-1 assay and Trypan Blue exclusion test. Effect of siRNA-induced knockdown of SORBS2 on cell viability was performed in a pool of RNA coming from many cellular types, whereas FISH is scored selectively in tumor cells. These findings suggest that TYMS and TYMP GCN and expression can be useful predictive markers of C sensitivity in human breast cancer.
in demographic characteristics, but the systemic endocrine profiles of Y and NY populations have not been studied, and may affect the generalizability of findings in studies of NAF.

Methods: A subset of participants from an ongoing case-control study was included (120 cases, 120 controls). Cases are women presenting to the Lynn Sage Breast Center with newly diagnosed unilateral breast cancer, and controls are women presenting to the Lynn Sage Mammography Center who do not have cancer. NAF collection was attempted on all consented subjects; those yielding ≥2 uL NAF were designated as Y and those yielding <2 uL NAF were NY (120 Ys, 120 NYs). Menopausal status, age, race, menstrual cycle phase, mammographic density, and serum hormone levels were also collected. Differences between Y and NY groups were tested using a t-test for age, \( \chi^2 \) for race, and Wilcoxon Sum Rank Test for serum hormone levels. Mammographic density was quantitated using digital or digitized images and the CUMULUS software. Linear regression was used to test the relationship between a logged serum hormone values and if a patient yielded adjusting for phase of menstrual cycle in premenopausal women.

Results: Mean ages of Ys and NYs were not different 51.0 and 52.6, respectively (p=0.11). The distribution of Ys by race (Caucasian, African American, other), was not different (\( \chi^2 \) p=0.58). Median serum prolactin concentrations were higher in Ys than NYs for both pre- (7.0 and 2.5 ng/ml) and post-menopausal (5.6 and 2.4 ng/ml) women, respectively (both p<0.01). Median serum estradiol was lower in yielding premenopausal patients only (pre- 90.5 and 64.3 pg/ml p=0.02; post- p=0.59). Stratifying the premenopausal women further into cases and controls, the difference only held for cases and not for controls (p=0.02, p=0.033). In the premenopausal patients the regression models suggested that these relationships for estradiol and prolactin remain even after adjusting for phase of the menstrual cycle. No differences in Ys versus NYs were found for progesterone, follicle stimulating hormone, or sex hormone binding globulin. Mammographic density was not different for Ys as compared to NYs for pre- (22% and 20%, p=0.83), but was marginally lower for post-menopausal Ys versus NYs (11% and 15% density; p=0.07). Following stratification of the postmenopausal women into cases and controls, the cases who yielded had significantly lower than NYs (11% and 19% density, p=0.03), but the control Ys and NYs were not different (10% and 11% density, p=0.65).

Conclusion: Pre- and post-menopausal women who yield NAF display increased serum prolactin levels, whereas decreased estradiol levels were observed in premenopausal NAF Ys. These findings suggest that NAF yield has systemic endocrine determinants, with implications for biomarker research on NAF samples. Further characterization of Y and NY women is important, along with standardization of methods of NAF collection, since NAF yield varies widely between studies.

P5-01-10

A Potential New Marker for, and Facilitator of, Hormone Independence.

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Triple negative breast cancers (TNBC) are generally more aggressive and have a worse prognosis than cancers that express estrogen (ER), progesterone (PGR) and/or human epidermal growth factor receptor 2 (HER2). Recent data suggests that another hormone receptor, that for androgen (AR) may be relevant in terms of further stratifying TNBC, with quadruple negative breast cancer (QNBC) having a worse prognosis than TNBC. In addition, TNBC vary with respect to prognosis and sensitivity to specific treatments, and foreknowledge of treatment outcomes has been the goal of numerous studies designed to generate predictive gene signatures. Development of these signatures involves the use of algorithms that can potentially exclude important biomarkers that may appear to be too weakly expressed to make the cut-off. We report here that the lipid metabolic enzyme, long-chain fatty acyl-CoA synthetase 4 (ACSL4) falls into this category of a heretofore-unrecognized biomarker for aggressive breast cancer. We have previously reported that ACSL4 expression is negatively correlated with the expression of ER and AR in both breast cancer cell lines and tumor samples. We now report a positive correlation between ACSL4 mRNA expression and QNBC status. In two separate studies involving a total of 71 separate breast cancer cell lines, values for ACSL4 expression derived from microarray data indicated increased expression in QNBC cell lines, p=3.75E-08 and p=4.59E-08, when compared with cell lines expressing one or more receptor biomarkers. An analysis of data derived from 178 tumor samples indicated a similar correlation, p=2.2E-03. ACSL4 expression was also correlated with molecular subtype. When breast cancer cell lines were classified by subtype, 24 of 26 luminal cell lines were negative for ACSL4 expression, 8 of 8 claudin-low cell lines were positive for ACSL4 expression, as were 12 of 17 basal-like cell lines. Three basal-like cell lines expressed both ACSL4 and HER2. Upon further examination, these lines were demonstrated to express relatively low levels of other markers that have been suggested to correlate with HER2 sensitivity (ESR1, XBPI, FASN, ERBB3), as well as to express relatively high levels of markers correlated with HER2 resistance (CD44, CAV2).

In order to determine the relationship between ACSL4 expression and estrogen independence, ACSL4-negative MCF-7 cells were inducibly transfected with ACSL4 cDNA. Induction of ACSL4 resulted in abrogation of the proliferation response to estrogen, accompanied by a reduction in expression of ER, AR and PGR. In addition, induced cells were less sensitive to treatment with tamoxifen, etoposide and triacsin C. Information derived from RAF-1-transfected MCF-7 microarray data suggest that ACSL4 expression can be regulated by RAF-1. To further explore this notion, QNBC cells, MDA-MB-231, which express high levels of ACSL4, were treated with siRNA for RAF-1 and ACSL4 levels monitored by RT-PCR and immunoblot. The fall in levels of expression of RAF-1 was accompanied by a similar decrease in expression of ACSL4. Reduction of ACSL4, however, had no effect on RAF-1 expression. In summary, these data demonstrate a possible role for ACSL4 as a biomarker for prognosis and treatment outcome, as well as a facilitator of hormone resistance.

P5-01-11

Small Node-Negative (T1b-cN0) Invasive Hormone Receptor (HR)-Positive Breast Cancers: Is There a Population Which Might Have Benefit from Adjuvant Chemotherapy?

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Background: It has been widely accepted that small and node-negative breast cancers have an excellent prognosis and do not generally have clinical benefit from adjuvant chemotherapy. Recently, the role of adjuvant chemotherapy for small node negative breast cancers has been justified in some high-risk patients, which include HER2-positive and triple negative breast cancers. However, the question has been raised as to whether there are some patients who might have benefit from adjuvant chemotherapy in small node-negative HR-positive breast cancers. According to the current 2011 NCCN guideline, 21-gene RT-PCR assay can be considered for tumor
size of more than 0.5 cm in HR-positive, HER2-negative cancers. In cases of high recurrence score (≥ 31), adjuvant chemotherapy in addition to endocrine therapy is recommended as category 2B. Because gene array cannot routinely be used in clinical practice and has not been validated in prospective randomized trials and the usefulness of it still needs to be defined, it would be better if there were valuable markers to determine risk for relapse in this setting. We hypothesized that there could be a population who might have clinical benefit from adjuvant chemotherapy in this small node-negative HR-positive tumors.

**Patients and Methods:** We retrospectively analyzed the clinicopathologic characteristics and outcomes of 538 postoperative HR-positive (ER-positive and/or PgR-positive) T1b-cN0 breast cancer patients between 2004 and 2007 at the Samsung Medical Center. We performed Cox regression multivariate analysis for relapse using variables from univariate analysis by log-rank test for relapse.

**Results:** The median age at diagnosis was 46 years (range, 22-79). During the median 60.5 months of follow-up, the 5-year recurrence rate was 5.2%. Anthracycline-based adjuvant chemotherapy was administered to 44.8% of the patients. Adjuvant endocrine and radiation treatment were administered to 94.6% and 63.7% of the patients. There were significant differences according to histologic grade (HG), Ki67 index, and age of less than 35 years in univariate analyses regarding RFS (p=0.003, p=0.0001, and p=0.003, respectively by log-rank test). There was no significant difference according to tumor size of subcentimeter (<1 cm) (p=0.826). In Cox regression multivariate analysis, high Ki67 index and young age of less than 35 years were identified as independent risk factors for relapse (p<0.0001 for Ki67 index and 0.015 for young age). The high risk patients (n=24, 4.5%) who have high Ki67 index (more than 75%, +) or young age of less than 35 and more than 50% of Ki67 index showed better RFS with statistical significance for anthracycline-containing adjuvant chemotherapy (p=0.029).

**Conclusion:** A patients’ population may exist who have clinical benefit from adjuvant chemotherapy in T1b-cN0 HR-positive breast cancer patients. Ki67 index and age are useful as valuable surrogate markers to predict recurrence and to have benefit from adjuvant chemotherapy in this population.

**P5-01-12**

Identification of an ATM Activation Subtype in PTEN Mutant Breast Tumours.


**Background**

PTEN is frequently lost in cancer cells through genetic mutation or epigenetic silencing. Loss of PTEN function has been widely reported to cause up-regulation of the PI3K/AKT signalling pathway resulting in increased cell growth, proliferation and survival. More recently it has been reported that PTEN null cells demonstrate genomic instability through increased ROS and oxidative stress induced DNA damage. The aim of this study was to identify a biomarker for PTEN status in human breast cancers.

**Materials and Methods**

A metagene representing ATM activation was generated from public cell line data of AT fibroblasts treated with gamma-irradiation. This was used to perform hierarchical clustering analysis of a public DNA microarray profiling dataset with known PTEN IHC status. The metagene was validated in PTEN wildtype and null breast cancer cell lines.

**Results**

We found that PTEN null cells have elevated levels of ROS and furthermore activation of the DNA damage signalling kinase, ATM. In agreement with this, the ATM metagene signature correlated with PTEN mutation in breast cancer tumours. Scoring of PTEN wildtype and null breast cancer cell lines using the metagene correlated with ATM activation and sensitivity to inhibition of ATM. Furthermore we show that inhibition of ATM caused DNA damage, cell cycle arrest and apoptosis in PTEN deficient cells suggesting a novel therapeutic strategy.

**Conclusion**

These observations suggest that ATM may represent a therapeutic target in PTEN deficient tumours and furthermore ATM activation may also be an important biomarker of PTEN mutation or loss in breast cancer.

**P5-01-13**

High Levels of Nuclear Heat Shock Factor 1 (HSF1) Are Associated with Poor Prognosis in Breast Cancer: Results from the Nurses’ Health Study.

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**Purpose:** Heat shock factor 1 (HSF1) is the master transcriptional regulator of the cellular response to heat and a wide variety of other stressors. We previously reported that HSF1 promotes the survival and proliferation of malignant cells. At this time, however, the clinical and prognostic significance of HSF1 in cancer is unknown.

**Patients and methods:** Breast cancer samples from 1,841 participants in the Nurses’ Health Study (NHS) were scored for levels of nuclear HSF1. Associations of HSF1 status with clinical parameters and survival outcomes were investigated by Kaplan-Meier analysis and Cox proportional hazard models. The associations were further delineated by Kaplan-Meier analysis using publicly available mRNA expression data.

**Results:** Nuclear HSF1 levels were elevated in ~80% of in situ and invasive breast carcinomas. In invasive carcinomas, HSF1 expression was associated with high histologic grade, larger tumor size, and nodal involvement at diagnosis (P<0.0001). Overall, in multivariate analysis, high-HSF1 levels were associated with increased breast cancer-specific mortality (HR, 1.62; 95% CI, 1.21-2.17). This association was seen in the ER-positive population (HR, 2.10; 95% CI, 1.25-2.47), even in early-stage lymph node negative cases (HR, 1.98; 95% CI, 1.17-3.33). In public expression profiling data, high-HSF1 mRNA levels were also associated with an increase in ER-positive breast cancer-specific mortality.

**Conclusions:** Increased HSF1 is associated with reduced survival in breast cancer. The findings indicate that HSF1 should be evaluated prospectively as an independent prognostic indicator in ER-positive breast cancer and that HSF1 may provide a useful therapeutic target.
P5-01-14
Phosphoproteomic Analysis of TIMP-1 Overexpressing MCF-7 Human Breast Cancer Cells Reveals Increased Expression and Phosphorylation of Topoisomerase Proteins.
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Background: Tissue inhibitor of metalloproteinase 1 (TIMP-1) is a protein with a potential biological role in drug resistance. Lack of TIMP-1 protein either alone (Willemoe et al., Eur J Cancer 2009) or in combination with Topoisomerase 2A gene aberrations has been shown to associate with increased benefit from adjuvant treatment with a Topoisomerase 2 inhibitor (epirubicin containing combination chemotherapy) while this association was not observed in patients treated with a non-Topoisomerase 2 inhibitor combination chemotherapy (Ejlsretsen et al., JCO 2010).

Aim: To further investigate the molecular mechanisms underlying the association between TIMP-1 and epirubicin sensitivity by quantitative phosphoproteomics.

Methods: MCF-7 human breast cancer cells were transfected with pcDNA3.1(Hyg)-TIMP-1. Among 11 single cell clones, two TIMP-1 low expressing and two TIMP-1 high expressing clones were selected. The clones were labeled by SILAC (stable isotope labeling with amino acids in cell culture). Lysates were digested with trypsin and fractionated with SCX followed by subsequent enrichment of phosphopeptides by TiO2-based chromatography and desalting by C18 purification. Total peptides and phosphopeptides were analyzed by tandem mass spectrometry and quantified as described (JV Olsen et al., Science Signaling 2010). Selected proteins were confirmed on Western blots. The sensitivity of the four TIMP-1 cell clones was analyzed by treatment of the cells with the following drugs: The Topoisomerase 2 inhibitor epirubicin (an anthracycline). The Topoisomerase 1 inhibitor SN-38 (the active metabolite of irinotecan, a camptothecin analogue) and the combination of these. A specific Topoisomerase 2B inhibitor (XK 469, a quinoxaline analogue and the DNA crosslinker cisplatin. All experiments were determined with an endpoint MTT assay.

Results: The quantitative proteomic analyses confirmed the differences in TIMP-1 levels among the four clones. Several proteins were consistently found to be upregulated and/or had changed phosphorylation levels in the TIMP-1 high cells in two biological replicates. Of particular interest was the observation that the phosphorylation status and protein levels of Topoisomerase-1, -2A and -2B were increased in TIMP-1 high expressing cells compared to TIMP-1 low expressing cells. When the four clones were treated with specific Topoisomerase inhibitors, the TIMP-1 high expressing cells exhibited significantly more resistance to all three inhibitors compared to TIMP-1 low expressing cells. When cells were treated with a combination of SN-38 and epirubicin, we observed an additive but not a synergistic effect. At last, cells were treated with cisplatin with no different effect on TIMP-1 high and low expressing cells.

Conclusion and perspectives: The observed upregulation of both protein and phosphorylation levels of Topoisomerasers in TIMP-1 high cells may be part of the mechanism by which TIMP-1 confers resistance to treatment with Topoisomerase inhibitors in primary breast cancer. Further work will include pathway analyses and hypothesis testing in clinical material.

P5-01-15
The Functional Role of the Estrogen-Regulated Gene GREB1: Characterization of a Novel GREB1 Knockout Mouse Model.
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Background: Gene regulated in breast cancer 1 (GREB1) was initially discovered in breast cancers as an estrogen-regulated gene that mediates estrogen-stimulated cell proliferation and is a candidate clinical marker for response to endocrine therapy. However, little is known of the functional role of GREB1 protein in normal breast tissue or breast cancers.

Methods: To address this unknown role, our laboratory designed and created a novel Greb1 Knockout Mouse model (C57/bl MEL Greb1 KO). This constitutive model results in the loss of Greb1 mRNA and protein expression in cells where expression of Cre recombinase promotes the cleavage of exon 1 and intron 1 of the gene encoding Greb1. ROSA26 Cre C57/bl MEL Greb1 KO mice heterozygous for the floxed Greb1 allele were crossed to generate experimental litters. Initial experiments were designed to evaluate if the complete loss of Greb1 expression in offspring homozygous for the floxed Greb1 allele was lethal during gestation. Experimental litters were tail clipped and genotyped using gDNA and genotype-specific PCR.

Results: Offspring homozygous for the floxed Greb1 allele were identified in expected Mendelian ratios with wild type and heterozygous siblings. Loss of Greb1 expression was confirmed using RT-PCR, in situ hybridization and immunoblotting. Loss of both Greb1 alleles was not observed to be lethal during gestation for either male or female pups. Preliminary gross observation of these homozygous KO mice revealed no overt anatomical differences, however, they were 25-30% smaller than their heterozygous and wild-type siblings. Breeding experiments are underway to determine the fertility of crossbred Greb1 homozygous KO mice. Imaging experiments and necropsy with histochemical analysis of tissues will reveal any alteration in architecture and function. These findings will be summarized in this presentation.

Discussion: As GREB1 has been identified as an estrogen-regulated gene involved in breast cancer cell proliferation and a potential target for new therapeutic strategies, it is important to understand the contribution of GREB1 to the differentiation, development and function of normal tissues as well as in breast cancers. Characterization of this novel Greb1 KO mouse model will provide answers to these functional questions surrounding GREB1.

P5-01-16
The Detection of Circulating CK19 mRNA-Positive Cells in the Blood and the Mitotic Index of the Primary Tumor Have Independent Prognostic Value in Early Breast Cancer.
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Background: Previous studies have shown that the molecular detection of CK-19 mRNA in peripheral blood and the mitotic index of primary tumor have prognostic value in early breast cancer. The aim of the present study was to assess the association between these variables. Patients and Methods: The primary tumors of 223 operable breast cancer patients (92 premenopausal and 131 postmenopausal) were evaluated for the mitotic activity index (MAI) classified either as <5/10, 6-10/10 and >10/10 or <10/10 and >10/10 mitoses/hpf using a standardized protocol as previously reported (Baak JP et al, Breast Cancer Res Treat 2009, 115:241-254). Peripheral blood was also
collected before the start and after the end of adjuvant chemotherapy for detection of CK-19 mRNA-positive cells by RT-PCR as previously described (Stathopoulou et al., Clin Cancer Res 2003, 9:5145–5151). Results: After a median follow-up of 118 months, 75 (33.6%) patients have experienced disease relapse and 56 (25.1%) have died of breast cancer. MAI was strongly associated with disease-free survival (DFS) and overall survival (OS) (p < 0.001 for both DFS and OS). Detection of CK19 mRNA-positive cells in the peripheral blood before but not after adjuvant chemotherapy was marginally associated with worse DFS (p = 0.055) and OS (p = 0.059). There was no correlation between MAI and CK19 mRNA detection before chemotherapy. Cox regression analysis revealed that both MAI and CK19 mRNA-positive cell detection before adjuvant chemotherapy were independent variables associated with decreased DFS (p < 0.001 and p = 0.038, respectively) and OS (p < 0.001 and p = 0.029, respectively). The interaction test showed no significant association between MAI and detection of CK19 mRNA-positive cells. Conclusion: MAI of the primary tumor and detection CK-19 mRNA-positive cells in the blood before the start of adjuvant chemotherapy in women with early breast cancer are two independent prognostic factors associated with clinical outcome.

P5-01-17
HER2 Amplification in Primary Tumor: A Potential Marker for Presence of Circulating Tumor Cells in Inflammatory Breast Cancer Patients?
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Background: Inflammatory breast cancer (IBC) is a rare but aggressive form of invasive breast cancer accounting for 3-6% of all cases and have higher rates of distant recurrence. Circulating tumor cells (CTCs) are known to predict outcome in metastatic breast cancer (BC) patients, but little is known about their prognostic significance in non-metastatic BC. We hypothesized that CTCs can be identified in patients with IBCs and may correlate with primary tumor characteristics. Methods: All patients had blood samples collected at the time of primary surgery. CTCs (per 7.5 ml blood) were detected using the Cell Search™ system (Veridex) and were defined as nucleated cells lacking CD45 but expressing cytokeratins (CK) 8, 18, or 19. The presence of ≥1 epithelial cells meeting morphologic criteria for malignancy was considered a positive result. Statistical analyses employed Chi square and Fisher’s exact tests using STATA software. Results: We prospectively evaluated 41 IBC patients enrolled in an IRB approved protocol undergoing surgery for stage I-III breast cancer. Median follow-up was 30 months. Mean age was 52 years. Thirty five patients (94%) had positive lymph nodes (LNs) at presentation, 30 (75%) had high-grade tumors and 20 (53%) had lymphovascular invasion. Eleven patients (28%) were ER positive, 11 (27%) were PR positive and 18 (44%) were HER2 positive. IBCs were more likely to be high grade (P < 0.0001), ER negative (P < 0.0001), PR negative (P < 0.0001), HER2 positive (P < 0.0001), High Ki-67 (P = 0.005) and had a BMI of more than 25 kg/m2 (P = 0.04). Twelve (27%) patients were CTC positive. CTCs were more likely be found in HER2 positive (8/18; 44%) vs. HER2 negative primary tumors (3/20; 15%) [OR = 4.53; 95% C.I. = 1.02-19.52; P = 0.04]. We found no statistically significant correlations between primary tumor characteristics (ER, PR, LNs, high grade) and presence of CTCs. Conclusions: About a quarter of IBC patients had CTCs at the time of primary surgery. In these patients HER2 overexpression predicted the presence of CTCs. Studies with longer follow-ups is needed to determine if CTCs predicted survival.

P5-01-18
Identification of Pax6 as a Human Breast-Cancer-Testis Antigen.
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Background:
Cancer-testis antigens (CTAs) are specifically expressed in the normal testis and various types of cancer cells, but not or expressed with very low level in the other normal tissues. CTAs are considered to be promising targets for immunotherapy. Little is known on CTAs which specifically expressed in breast cancer. Studies show that the transcriptional activation by CpG island hypomethylation is believed to be an important mechanism of their increased expression.

Objective:
To find CTAs which specifically expressed in breast cancer; prove the increase of its expression in human breast cancer, and explore the possibility for it to be developed as target for the immunodiagnosis and immunotherapy of breast cancer.

Methods and results
Genomic DNAs were isolated from testis tissue, breast cancer and normal breast tissues respectively. To perform CpG island microarray assays, genomic DNAs were prepared from 15 paired breast cancer biopsies and their normal controls. After obtaining sequence information for those clones from the CpG island database, they were then used to search for surrounding genes in the gene banks. We found several testis specific genes, such as, Pax6, NDRG3 and Synaptophysin. Since the last two genes had been extended investigated, they were not the subjects of this study. The Pax6 Gene Was Activated in Established Primary Breast Cancer Cell Lines. First, we examined Pax6 gene expression levels in a breast cancer cell line panel that included Hs578T, MDA-231, MDA-436, MDA-468, T47D, MCF7 and MCF7/Adr, by performing RT-PCR where b-actin served as control. In addition, the RNAs obtained from normal breast and testis tissues were used as negative and positive controls. We found that Pax6 was re-expressed in MDA-436, MDA-468, T47D, and MCF7/Adr. To further test the Pax6 gene’s behavior in primary breast cancer cells, we isolated breast cancer epithelial cells from the effusions of 8 patients (E4, E5, K151, K259, K473, K573, K596 and K605), cRNAs were prepared from those cells and the normal human breast cell line (HNBEC) for RT-PCR assays, where cRNAs of paired MCF7 cells and b-actin served as controls. Results showed that Pax6 was expressed in K259, K573, K596 and K605 cells to various degrees. Pax6 was re-expressed in a majority of Breast Cancer Biopsies. To test if Pax6 was also expressed in breast cancer tissues, IHC assays were carried out using 19 patients’ biopsies where human testes served as positive controls. We found that the Pax6 protein existed mainly in the cytoplasm of breast cancer cells, but was not detectable in normal breast tissue sections.

Conclusion
CpG islands of Pax6 promoter region were hypomethylated in human breast cancer. The mRNA level of Pax6 in human breast cancer was significantly increased contrast to normal breast tissue, contrast to all other analyzed normal tissues, suggesting Pax6 is a CTA for breast cancer and might be applied as molecular target in immunotherapy against human breast cancer.
P5-01-19
Endothelin-1 Expression in Breast Cancer Tissue, Surrounding Stroma, Correlation with Tumor Microvessel Density and Clinical Outcome.
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Background: Endothelin-1 (ET-1) is a peptide which regulates normal biological processes such as vascular tone. In addition, endothelin signaling pathway is dysregulated in pathophysiological conditions such as cancer and fibrosis. It has been shown that endothelin-1 is expressed in breast cancer tissue but little is known with regard to ET-1 expression in surrounding tumor stroma. In the present study, we investigated ET-1 expression in breast tumor cells, surrounding stroma, association with tumor microvessel density (MVD) and impact on clinical outcome.

Materials and Methods: We conducted a retrospective, multicenter study. Patients from 3 medical centers with histologically documented stage I-III invasive breast cancer were included in the study. Paraffin embedded formalin fixed breast cancer tissue and surrounding stroma were evaluated for ET-1 and CD34 (marker for MVD) by IHC. ET-1 cytoplasmic expression was scored as positive (3+ by IHC) or negative (0, 1+, 2+). Stained vessels by CD34 were counted in five consecutive fields at 40 x magnification and their mean was recorded. Demographics, clinical data and recurrence free interval (RFI) in months were available for statistical analysis.

Results: The study included 92 patients with average age of 55 years at diagnosis. Median follow up of patients at the time of analysis was 72 months. Total of 29 patients experienced disease progression (17 locoregional and 12 distant). Tumor ET-1 expression positively correlated with earlier stage: odds ratio (OR) = 22 for stage I and OR=20 for stage II as compared with stage III. Positive ET-1 staining in tumor was detected in 72.8 % of cases, while ET-1 positive expression in stroma was detected only in 6.5% of cases. Interestingly, all ET-1 stroma positive tumors were estrogen receptor (ER) positive. The means of CD34 were not different according to ET-1 expression either in the tumor or stroma. Triple negative breast cancer tumors exhibited higher MVD (p=0.0177). In the logistic regression model relating ET-1 expression in tumor to clinical variables, ET-1 positive tumors showed a trend for the association with higher relapse rate (p=0.058). Multivariate analysis suggested that there was no significant difference in the recurrence free interval (RFI) between the ET-1 positive and ET-1 negative groups (long-rank test p-value = 0.71). Survival analysis identified stage as a significant predictor of RFI in the Cox proportional hazard model that included ET-1 expression and other clinical variables.

Conclusions: The significant predictor for ET-1 expression in the tumor was early stage. High tumor ET-1 expression was more common in patients who experienced breast cancer recurrence. No association was found between ET-1 expression and MVD, and between ET-1 expression and time to recurrence. Further studies with larger sample size are needed to better delineate a role of ET-1 as prognostic biomarker in early stage breast cancer.

P5-02-01
ErbB4 Ectodomain as a Biomarker and a Potential Therapeutic Target for Breast Cancer.
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ErbB4 function is controversial in breast cancer and influenced by alternative splicing of the ERBB4 gene. Here we evaluated the cleavable ErbB4 JM-a isoform as a potential drug target and biomarker. To address ErbB4 cleavage in breast cancer tissues in vivo, extracellular and intracellular cleavage products of ErbB4 were assessed by ELISA and immunohistochemistry using matched serum and tissue samples from a series of 243 breast cancer patients. Elevated serum ectodomain level (≥40 ng/mL) and nuclear immunoreactivity were present in 21% and 52% of the patients, respectively. An elevated serum ErbB4 ectodomain concentration was not associated with tumor nuclear immunoreactivity, but was significantly associated with the premenopausal status at diagnosis (P = 0.04). Estradiol also enhanced ErbB4 cleavage and ErbB4 cleaving enzyme activity in vitro, supporting a role for estrogen signaling in regulation of ErbB4 cleavage. Selective targeting of the cleavable ErbB4 JM-a in human breast cancer cells with an ErbB4 isoform-specific mAb 1479 inhibited ErbB4 cleavage and reduced tumor formation in a mouse xenograft model. Consistent with blocking of ErbB4 cleavage, mutagenesis studies and the 3.4 Å X-ray crystal structure of a complex of the ErbB4 extracellular region and the 1479 Fab localized the binding site of mAb 1479 on ErbB4 to a region on subdomain IV encompassing JM-a-specific residues. These data demonstrate that ErbB4 is cleaved in a subset of breast cancer patients, and suggest that the mechanisms by which mAb 1479 suppresses breast cancer cell growth involves inhibition of ErbB4 cleavage. Serum ErbB4 ectodomain concentration could be used as a biomarker to monitor the activity of compounds, such as mAb 1479, that target ErbB4 cleavage.

P5-02-02
Divergent Effects of Zoledronate and Denosumab on Cellular Interactions between Osteoblasts and Breast Cancer Cells.
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Background: The development of bone metastases is a multistep process that involves interactions between tumor cells and normal host components of the bone marrow microenvironment. Emerging evidence suggests that the interplay of breast cancer cells with both RANKL-expressing osteoblasts, key mediators of osteoclastogenesis, and osteoblast-derived extracellular matrix structures is critical to the metastatic process. This relationship may contribute to the tumor cells’ colonization of bone, invasive behavior, and eventual tumor progression. Zoledronate, a nitrogen-containing bisphosphonate has demonstrated inhibitory effects on tumor cell adhesion and migration. Denosumab, a fully humanized monoclonal antibody against receptor activator of nuclear factor-κB ligand (RANKL), might also exhibit inhibitory effects on breast cancer cell adhesion and migration by neutralizing RANKL. However, despite their clinical importance, the mechanisms of zoledronate and denosumab in crosstalk between breast cancer cells and osteoblasts still remain poorly characterized.
Material and Methods: To investigate the influence of zoledronate and denosumab on osteoblast-breast cancer cell interactions, cell adhesion and migration assays using different breast cancer cell lines (MCF7, MDA-MB-231, and SKBR3) were performed.

Results: Cell-matrix adhesion assays revealed a strong attachment of breast cancer cells to several extracellular matrix components expressed by osteoblasts. The subsequent incubation of attached cells with 100 µM zoledronate for 72 h resulted in a prominent reduction of tumor cells’ binding to collagen type I, fibronectin, defined laminin isoforms and tenasin-C, whereas incubation with 10 µg/ml denosumab did not affect the adhesive behavior of breast cancer cells. In addition, cell-cell adhesion analyses indicated that zoledronate was able to decrease the attachment of breast cancer cells to osteoblasts. Furthermore, migration studies using the scratch assay and the transwell migration technique clearly showed that the conditioned media of osteoblasts (primary osteoblasts as well as the osteoblastic cell line CAL72) strongly enhanced the migration of the breast cancer cell line MDA-MB-231 compared to the conditioned media of osteoclast-like cells, stromal cells and control medium, suggesting that exclusively osteoblasts release factors that significantly increase tumor cell migration. Interestingly, exposure to zoledronate inhibited osteoblast-induced tumor cell migration in a concentration-dependent manner. In contrast, denosumab (0.1 – 50 µg/ml) had no inhibitory effect on the migration of MDA-MB-231 cells in scratch analysis. However, this agent was found to cause a reduction in the number of migrated cells when 10 µg/ml were used in transwell assays.

Discussion: Our data clearly demonstrate that breast cancer cells interact with osteoblasts and osteoblast-secreted extracellular matrix proteins. The osteoblast-induced breast cancer cell abilities, including tumor cell adhesion and migration, were strongly affected by zoledronate, but not by denosumab. The influence by zoledronate on these interactions provides further insights into the antiresorptive effect of this substance on bone metastasis.

P5-02-03
Lipid Raft Disruption by Docosahexaenoic Acid Induces Apoptosis in Transformed Human Mammary Luminal Epithelial Cells Harboring HER-2 Overexpression.
Ravacci GR, Tharcisio Junior T, Logullo AF, Torrinhas RS, Brentani MM, Waisberg DL. Medical School, University of São Paulo, São Paulo, Brazil; UNIFESP University, São Paulo, Brazil

Background, In HER-2 overexpressing breast cells, HER-2 receptors exist on the cell surface as monomers, homodimers and heterodimers with EGFR and HER-3. For signal activation and transduction to occur, the HER-2 receptors must be located in lipid rafts. Therefore, HER-2 overexpression must be accompanied by increased lipid rafts on the cell membrane to ensure constant signal transmission. Methods, To test this hypothesis, we used HB4a – untransformed human mammary epithelial cell strain – and HB4aC5.2 cells – HB4a cells co-transfected with ErbB-pJ5E.c-2 (normal full length human HER-2 cDNA). We analyzed the lipid rafts, cholesterol and fatty acids from cell membrane by confocal microscopy and gas chromatography. The proteins associated to HER-2 activity and lipid rafts formation, such as Akt, ERK ½ and FASN were analyzed by western blotting. To evaluate the lipid rafts importance for HER-2 signaling, we treated cells with docosahexaenoic acid (DHA) to disrupt the lipid rafts. Thereby, all analysis was repeated and cells death and apoptosis were analyzed by flow cytometry. Values were obtained by the mean and standard deviation of five independent experiments and developed in triplicate, by using one-way analysis of a variance (ANOVA) test, followed by Bonferroni post-test, to identify significant (p<0.05) differences between cell lines and results with different concentrations of DHA treatment. Results, HB4aC5.2 cells have five copies of pJ5E.c-ErbB-2 inserted into their genome and express approximately 900 times more HER-2 than HB4a cells. HER-2 overexpression in HB4aC5.2 cells was accompanied by increased lipid rafts in cell membranes, hyperactivation of downstream Akt and ERK1/2 proteins, and rate of cell growth increase as compared to the HB4a strain. In addition, HER-2 overexpression was associated to increased activation of FASN, a key enzyme involved in cellular lipogenesis. The final product of FASN activation, palmitate, is used to synthesize saturated phospholipids, necessary to form lipid rafts. Treatment of HB4aC5.2 cells with DHA, an omega-3 fatty acid, disrupted the lipid rafts by its increased incorporation into the cell membrane; inhibited HER-2 activation by decreasing Akt, ERK1/2, and FASN proteins; and induced apoptosis. The same treatment maintained the structure of HB4a cell lipid rafts, accompanied by a low percentage of DHA-induced apoptosis. Conclusion, Although little is yet known about lipid rafts, our data support the theory that disturbances these micro-domains may represent a useful tool in controlling cell signaling triggered by HER-2 receptors and contribute to a better understanding of the role of this receptor in HER-2 positive breast cancer.

P5-02-04
Disruption of Endothelial Cells Barrier Integrity by Invasive Breast Cancer Cells.
Haidari M, Zhang W, Chen Z, Mortazavi A, Dixon R. Texas Heart Institute, Houston, TX; The University of Texas Health Science Center at Houston, Houston, TX

Despite its critical role in cancer metastasis the molecular mechanisms regulating breast cancer cells transendothelial migration are poorly understood, but clearly depend on the invasive capacity of tumor cells and their ability to breach the endothelial cell barrier. Vascular endothelial-cadherin (VE-cadherin) is found specifically in the endothelial cell adherens junction and has been implicated in playing a fundamental role in controlling the transport across the endothelial barrier. Tyrosine phosphorylation of VE-cadherin has been implicated in the disruption of endothelial cells adherens junctions and diapedesis of metastatic cancer cells. We tested this hypothesis that interaction of breast cancer cells with endothelial cells initiates the signal transductions that disrupt the endothelium barrier integrity. Our studies demonstrated that the attachment of MDA-MB-231 human breast cancer cells to Human Umbilical Vein Endothelial Cells (HUVECs) leads to tyrosine phosphorylation of VE-cadherin and the formation of gaps between endothelial cells. These were accompanied by activation of two tyrosine kinases, Src and proline rich tyrosine kinase (Pyk-2). In addition, immunoprecipitation studies indicated that the endothelial cells adherens junction structure was disrupted through MDA-MB-231-induced dissociation of VE-cadherin and β-catenin complex. Activation of RhoA and HRas by over expression of constitutively active forms of the genes leads to tyrosine phosphorylation of VE-cadherin and Pyk-2 in HUVECs. Over expression of dominant negative forms of RhoA, HRas, Raf and ERK2 but not Rac1 and Cdc42 attenuated breast cancer cell-induced tyrosine phosphorylation of VE-cadherin and Pyk-2 in HUVECs. Indicating that breast cancer cell-induced VE-cadherin tyrosine phosphorylation and disruption of adherens junction in endothelial cells is mediated by RhoA and Hras/Raf/MEK/ERK signaling cascade. Understanding the precise molecular mechanisms...
that facilitate breast cancer cells transendothelial migration could develop novel therapeutic strategies targeting cancer cell metastasis by improving the protective role of endothelial cells.

**P5-02-05**


Suetsugu A, Digman M, Gratton E, Moriwaki H, Saij S, Bouvet M, Hoffman RM. AntiCancer Inc., San Diego, CA; University of California San Diego, San Diego, CA; Gifu University Graduate School of Medicine, Gifu, Japan; University of California Irvine, Irvine, CA

Background: Paxillin is involved in the assembly of focal adhesions. We wish to visualize paxillin behavior in breast cancer cells in vivo, as well as in vitro.

Materials and Methods: Dual-photon confocal microscopy was used to image paxillin behavior by linking it to GFP. MDA-MB-231 human breast cancer cells expressing paxillin-GFP were imaged in vitro and in vivo adhering and trafficking in blood vessels in mice. 10° paxillin-GFP expressing breast cancer cells were injected in the epigastric cranialis vein.

Results: In vitro, round breast cancer cells had greater paxillin movement than stretched cancer cells as seen by fluorescence imaging. Paxillin-GFP breast cancer cells in the epigastric cranialis vein were initially rounded and had GFP-expressing protrusions. At later timepoints, many paxillin-GFP-expressing breast cancer cells stretched. The breast cancer cells then extravesated and subsequently grew around the outer surface of the blood vessel after one week. Two weeks after injection, paxillin-GFP expressing breast cancer cells were imaged migrating along the vessel wall. Most of the paxillin-GFP expressing breast cancer cells were stretched and were not mobile. With anti-VEGF treatment, paxillin was observed in round structures within the cells rather than stretched structures and paxillin movement within the cell was arrested.

Discussion: These results demonstrate that breast cancer cells brightly expressing paxillin-GFP and two-photon confocal microscopy can allow the visualization of the behavior of paxillin within breast cancer cells during adhesion and migration along the walls of blood vessels, as well as during anti-angiogenesis therapy.

**P5-03-01**

Cytoplasmic Cyclin E and P-CDK2 Expression in Triple Negative Breast Carcinomas Measured by Immunohistochemistry Correlates with Poor Outcome.

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Background: Triple negative breast carcinomas (TNBCs) are aggressive malignancies that lack effective therapeutic targets but express low molecular isoforms of cyclin E (LMW-E). These LMW-E, generated primarily from NH2-terminal elastase cleavage of full length cyclin E (EL), preferentially accumulate in the cytoplasm of cancer cells. Using a transgenic mouse model system, our laboratory has recently shown that cyclin-dependent kinase 2 (CDK2) is required for LMW-E-mediated mammary tumorigenesis. These results lead us to hypothesize that immunohistochemical cytoplasmic detection of LMW-E and phospho-CDK2 in TNBC provides a valuable screening tool for those patients most likely to have a poor prognosis who could then be treated with anti-CDK2 therapy currently clinically available.

Material and Methods: Tissue micro-arrays from 168 TNBC patients were IHC stained for cyclin E and p-CDK2. Cyclin E staining intensity and percentage of positivity were evaluated both in the nucleus and cytoplasm of cancer cells and four different phenotypes of cyclin E were distinguished with respect to predominant nuclear or cytoplasmic localization of staining: cyclin E negative, predominantly nuclear, both nuclear and cytoplasmic and predominantly cytoplasmic. p-CDK2 IHC was achieved using an antibody, which recognizes phospho-threonine 160 on CDK2. Immunoreactive scores were determined by multiplying the intensity with the extent of staining of nuclei and cytoplasm. We sought correlations between different cyclin E and p-CDK2 expression patterns and disease-free survival (DFS).

Results: Cytoplasmic cyclin E accumulation on IHC of TNBCs correlated with poor outcome. Within the median follow up of 7.3 years tumors with both nuclear and cytoplasmic cyclin E expression demonstrated higher recurrence rate compared to entirely negative for cyclin E (p=0.0117). In contrast patients with exclusively nuclear cyclin E showed only a trend toward decreased DFS compared to patients with cyclin E negative tumors (p=0.0896). Furthermore we identified the new phenotype of cyclin E immunoreactivity, which is characterized by negative nucleus and positive cytoplasmic staining. This phenotype was the most significantly associated with poor DFS compared to cyclin E negative phenotype (p=0.0026) and as the only one distinguished at high risk of early recurrence among TNBC patient without axillary nodes involvement (p=0.0105). The expression of p-CDK2 was significantly higher in this phenotype than the cytoplasmic cyclin E negative tumors. High p-CDK2 tumors were also correlated to worse DFS then p-CDK2 low tumors (P=0.019).

Lastly, our analyses revealed that tumors positive for both cytoplasmic cyclin E and p-CDK2 had higher recurrence rate compared to negative for both or positive for one of them (p=0.003).

Discussion: Cytoplasmic cyclin E may help to predict recurrence, especially in early stage, node negative TNBCs. We present a new concept in assessing cyclin E expression. Poor outcome due to TNBCs overexpressing LMW-E provide a rationale to investigate the treatment strategies that could specifically target high LMW-E tumors. These patients could particularly benefit from treatment with CDK2 inhibitors.

**P5-03-02**

Aurora A Is Closely Linked to an Aggressive Phenotype of Breast Cancer.

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(Purpose) Aurora A, a mitotic serine/threonine kinase, is involved in important process of mitotic progression. The aim of this study is to clarify characteristics of breast cancer with Aurora A expression. (Methods) We examined, by immunohistochemical analysis, the expression of Aurora A in primary invasive breast cancer. We also investigated the correlation between expression of Aurora A and clinicopathological and biological factors. In addition, we studied the prognostic value of Aurora A in breast cancer.

(Results) In 215 cases of invasive cancer examined, Aurora A was mainly identified in tumor nuclei. Aurora A expression was detected in 67 (31.2%) cases. Expression of Aurora A was correlated with tumor size (p=0.002), lymph node metastasis (p=0.002), tumor stage (p=0.04) and histological grade (p=0.0031). Aurora A expression was also associated with hormone receptor negativity (p=0.0001), and increased levels of HER2 (p=0.0028), Ki67 (p=0.0001), HIF-1alpha (p=0.0001), VEGF (p=0.007), COX-2 (p=0.002) and p53 (p=0.0001). Patients who had increased Aurora A levels in their tumor showed shorter disease-free survival (DFS) (p<0.0001) and overall survival.
(OS) (p=0.0002) than those lacking Aurora A in univariate analysis. However, in multivariate analysis of DFS and OS, Aurora A was not identified an independent prognostic factor.

(Conclusion) Aurora A expression was significantly correlated with tumor burden, poorly differentiation, negativity of hormone receptors, tumor cell proliferation, and tumor angiogenesis. These findings suggest that Aurora A was closely related with an aggressive phenotype in breast cancer.

P5-03-03
Identification of New Substrates for Breast Tumor Specific Low-Molecular-Weight Cyclin E Cyclin-Dependent-Kinase 2.
Akli S, Duong M, Keyomarsi K, M. D. Anderson Cancer Center; University of Texas, Houston, TX

Background: Cyclin E overexpression occurs in 25% of breast cancer tumors and is linked to poor prognosis. In tumor cells full length cyclin E (FL-E) is processed by an elastase-like protease into low-molecular weight isoforms (LMW-E) that are biochemically hyperactive. When overexpressed in breast cancer cells, LMW-E induced genomic instability and resistance to p21, p27, and antiestrogens. LMW-E are tumor specific and are associated with the aggressive triple negative breast cancer (TNBC). Transgenic mice overexpressing LMW-E had increased incidence of mammary tumors and distant metastasis when compared to FL-E. Furthermore, when the role of CDK2 in LMW-E mediated mammary tumorigenesis was interrogated by crossing LMW-E transgenic mice with CDK2 knock out mice, we found that the mice are protected against mammary tumor formation. Additionally, treatment of LMW-E transgenic mice with roscovitine (a CDK2 inhibitor) delays mammary tumor formation. These results indicate that kinase inhibition may have therapeutic activity in LMW-E overexpressing tumors as shown in this preclinical model. These results lead us to hypothesize that the biological and biochemical differences between FL-E and LMW-E may be due to the phosphorylation of a distinct set of substrates when complexes with CDK2. Our goal is to identify distinct LMW-E/CDK2 substrates on a proteome-wide scale that could serve as novel therapeutic targets for the treatment of the aggressive LMW-E expressing TNBC.

Material and Methods: We used two different approaches to identify LMW-E/CDK2 substrates. 1) We generated an analog sensitive CDK2 kinase, (F80A or F80G)-CDK2 to specifically radiolabel its substrates in cell extracts followed by their identification by mass spectroscopy. 2) We incubated ProtoArrayMicroarrays spotted with 3000 GST-tagged human protein on high density glass slides either with recombinant active FL-E/CDK2 or LMW-E/CDK2.

Results: In the first approach, we expressed and purified wild-type CDK2 in complex with FL-E or LMW-E and CDK2 (F80A) and CDK2 (F80G) from insect cells. Although all 3 CDK2 kinases can use ATP to phosphorylate GST-Rb protein, only the F80G mutant can use PE-ATP-p-S. In the second approach, we incubated incubated protein arrays either with recombinant active FL-E/CDK2 or LMW-E/CDK2 at a concentration of 50 nM. Our first screen identified a total of 122 potential substrates to both FL-E/CDK2 or LMW-E/CDK2 kinase complexes. We only identified 1 protein that is phosphorylated by FL-E/CDK2 significantly more than by LMW-E/CDK2 as compared to the 32 potential substrates specific to LMW-E/CDK2 suggesting that by losing the N-terminal portion, the LMW-E/CDK2 kinase complex is able to specifically interact and phosphorylate novel proteins.

Discussion: The identification of new physiological LMW-E/CDK2 substrates will lead to the development of novel targets for therapeutics and the identification of the biological function for the treatment of the aggressive LMW-E expressing TNBC.

P5-04-01
BP1, a Homeotic Transcription Factor, Indirectly Upregulates ER-alpha in ER Positive Cell Lines.
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Background: BP1 is a member of the homeobox gene family of transcription factors. Our recent studies have shown that BP1 may play a role in breast cancer cell survival, aggressiveness and metastasis. BP1 protein (pBP1) is expressed in 80% of invasive ductal breast tumors, including in 57% of ER positive and 89% of ER negative tumors. BP1 overexpression results in a more aggressive phenotype in breast cancer cells and larger tumors in women with
breast cancer. We also observed the presence of mammary tumors in mice without estrogen supplementation after injection of MCF7 cells overexpressing BP1 compared with unsupplemented mice receiving MCF7 cells containing an empty vector. Our goal was to determine the pathway by which BP1 might cause this decrease in estrogen dependence of MCF7 cells. ER-alpha is a nuclear hormone receptor activated by estradiol and is a major target of anti-estrogen therapy. It is tightly regulated at the genomic and the non-genomic levels. Increased stability of ER-alpha is one mechanism by which cells can become ER-alpha independent. One way of altering ER-alpha stability is via p300 and BRCA1 since p300 acetylates and stabilizes ER-alpha, while BRCA1 destabilizes ER-alpha. We have previously shown that BP1 transcriptionally down-regulates BRCA1, suggesting that pBP1 may participate in this pathway.

Materials and methods: Protein levels of BRCA1, p300, pS2, pBP1 and ER-alpha were determined by Western Blot analysis. RNA levels were measured by real-time PCR. A ChIP assay was performed to confirm binding of pBP1 to the EP300 gene and binding of ER-alpha to BP1. MCF7 parental cell lines were treated with 10nM E2 to induce the expression of pBP1. Experiments involving effects of siBP1 on p300 expression are underway.

Results: Our data demonstrate that MCF7 ER+ cells engineered to overexpress BP1 exhibit increased ER-alpha protein, increased p300 and decreased BRCA1 protein as compared with control cells containing an empty vector and grown under the same conditions. Increased ER-alpha levels correlated with increased pS2 expression, a known downstream target of ER, demonstrating that there is more functional ER-alpha protein in cells overexpressing BP1. ChIP assays showed that pBP1 binds in the first intron of EP300-Histone acetyltransferase (p300). ChIP assays also showed that ER-alpha binds to an estrogen response element (ERE) upstream of the BP1 gene. MCF7 parental cell lines, when serum starved and treated with E2, showed increased BP1 expression compared with cells grown in charcoal stripped serum. Thus, BP1 may increase the stability of ER-alpha by decreasing BRCA1 and increasing p300.

Conclusions: pBP1 binds and transcriptionally activates p300, leading to increased ER-alpha stability, while also partially repressing BRCA1. Moreover, ER-alpha, via a feed-forward mechanism, binds to the BP1 gene and increases its expression.

P5-04-02
TNBL: An Integrated Knowledge Space of Triple-Negative and Basal-Like Breast Cancers.
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Background: To facilitate drug discovery and development in triple-negative (TNBC) and basal-like (BLBC) breast cancers, an integrated knowledge space was developed through standardizing and linking information reported in scientific publications. The tool was designed to 1) track and consolidate knowledge around TNBC and BLBC; 2) centralize computational resources on molecular profiles, disease models, drug effects and clinical outcomes; 3) establish a computational platform to facilitate identification of drug targets and biomarkers; 4) enable hypothesis generation for novel uses of pre-existing compounds including new indications and combinations. Technology and robust visualization capability, it offers integrated and exchange knowledge efficiently. Through its powerful semantic information from all possible sources in a computable format.

Discussion: The number of research papers on TNBC and BLBC are rapidly growing with 30-50% increase annually. TNBL provides a platform for the research community to standardize scientific findings and exchange knowledge efficiently. Through its powerful semantic technology and robust visualization capability, it offers integrated and comprehensive views of TNBC and BLBC which are not possible in the scope of any single research paper. In addition, it facilitates high quality hypothesis generation through consolidating up-to-date information from all possible sources in a computable format.

P5-04-03
Personalized Cancer Treatment Selection Using Computational Signaling Pathway Models.
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Introduction: In clinical oncology, with breast cancer in the front line, - cancer genome sequencing is entering clinical diagnostics; - novel drugs target signaling pathways that drive tumor growth; - metastasized cancer is expected to change into a chronic disease, requiring therapy response monitoring and repeated personalized choice of targeted therapy. Hence, there is a need to clinically interpret available genomic data in order to diagnose which signaling pathway is driving tumor growth in a patient, including the causative mutation, such that appropriate targeted drugs can be chosen. Currently available pathway analysis tools and databases are not suitable for this.

Companion diagnostics: To address the above need, we are developing computational models of signaling pathways, which can be used as companion diagnostic tools to translate genomic data into meaningful clinical information to enable selection of targeted drugs. The aim is to develop such models for the 10 to 20 most relevant oncogenic pathways, and use them to predict which one is most likely to drive tumor growth in an individual patient, including the probable underlying genomic defect.

A model of the Wnt pathway: As a proof of concept, we have built a computational model of the Wnt pathway, which is active in colon...
adenoma and a main player in colon cancer, but also relevant in breast cancer. Our first generation model of the Wnt pathway covers its transcriptional program, for which we carefully selected a list of Wnt target genes. We have modeled this pathway by a Bayesian network, which interprets the expression levels of the target genes (from Affymetrix U133Plus2.0 arrays), and infers a probability that the Wnt pathway is active in a certain patient tumor sample. The parameters of the model are partly derived from literature and partly fitted on experimental data.

**Results:** We first fitted a model on data from six pairs of Wnt knock-down experiments on colon cancer cell line LS174T, and tested it on a public set of 32 normal colon samples plus 32 colon adenomas from patients (GSE8671). This model perfectly predicted no Wnt activity in the normal samples and an active Wnt pathway in the adenomas. Next, we fitted the model on these 64 patient samples, and tested it on a set of 145 normal colon and colon cancer samples from patients (GSE20916). The model again predicted no Wnt activity in all 44 normal samples, and an active Wnt pathway in all cancer samples, except for four of the 36 samples from surgically removed colon carcinomas, which most likely are the most heterogeneous.

Thirdly, we took the latter model, trained on colon samples, and tested it on three breast cancer data sets, one with 51 breast cancer cell lines (GSE12777) and two sets of breast cancer samples from patients (GSE12276, n = 204; GSE21653, n = 266). Also on this different tissue type, our model correctly predicted Wnt activity for the few breast cancer cell lines that are known to have an active Wnt pathway. For the two patient studies, a significantly higher number of samples of the basal subtype were predicted to have an active Wnt pathway compared to the other subtypes (Fisher’s exact test: p = 0.021 for GSE12276; p = 2.7e-5 for GSE21653), in line with increasing evidence for Wnt activation in the basal subtype.

**P5-05-01**

**Vitamin D Status in Newly Diagnosed Breast Cancer Patients Inversely Correlates with Tumor Size and Moderately Correlates with Outcome.**


**Aims:** To study the impact of Vitamin D (VitD) status and genetic variability in key VitD regulatory genes on patient and breast tumor characteristics, and on breast cancer related outcome.

**Methods:** We examined serum 25-hydroxyvitamin D$_3$ (25OHD) levels in a cohort of 1800 early breast cancer patients treated in Leuven between 2003 and 2010. Serum was collected at diagnosis for all patients; germline DNA from peripheral blood was also available for the majority of them. Serum 25OHD was measured by radioimmunoassay, and single nucleotide polymorphisms (SNP’s) were assessed by Sequenom. Statistical analysis was done by multivariable regression models including age, BMI and season and by a Cox proportional hazard model for analysis of disease-specific survival and disease-free interval.

**Results:** Lower 25OHD serum levels were significantly correlated with larger tumor size (0.4 ng/ml decrease in 25OHD per 1 cm increment in tumor size, p=0.0063) but not with lymph node invasion, estrogen receptor and HER2 status, or tumor grade. Serum 25OHD level was significantly affected by genotypes of rs10741657 and rs1993116 (25-hydroxylase; CYP2R1) and of rs222040, rs7041 and rs4588 (D-binding protein; DBP), yet its observed association with tumor size did not differ between distinct genotypes. Although not statistically significant, patients with higher 25OHD levels tended to have slightly improved survival. Also, a significant increase in relapse risk became apparent after 3 years for patients with 25OHD<30 ng/ml.

**Conclusion:** Vitamin D Level in breast cancer patients inversely correlates with tumor size and moderately correlates with outcome.

**P5-05-02**

**Phenotypic Plasticity in the Normal Breast.**

Sauder CAM, Koziel JE, Choi M, Fox MJ, Badve S, Blosser RJ, Mathieson T, Rufenardt CA, Henry JE, Storniolo AMV, Herbert B-S, Clare SE. Indiana University School of Medicine, Indianapolis, IN; Susan G. Komen for the Cure® Tissue Bank at the IU Simon Cancer Center, Indianapolis, IN; The Catherine Peachy Fund, Inc., Warsaw, IN

Background: The cell of origin of metaplastic carcinoma of the breast (MCB) is an enigma. MCB comprises less than 4% of all breast cancers, and is a member of the subtype of breast cancer referred to as Triple Negative Breast Cancer (TNBC). It is aggressive with a prognosis worse than that for invasive ductal carcinoma NOS (not otherwise specified), as well as other TNBCs. Identification of the histogenesis of MCB is likely to provide clues to its oncogenesis. Differentiation along non-epithelial lineages is also observed in benign lesions of the breast. Adenomyoepitheliomas, thought to arise from myoepithelial cells, have been observed to contain areas of cartilaginous, sebaceous, squamous, and osseous differentiation.

**Methods:** Healthy woman volunteers from central Indiana were invited to donate breast tissue to the Susan G. Komen for the Cure® Tissue Bank at the IU Simon Cancer Center. Starting from the 10 gauge tissue cores, 28 normal mammary epithelial (HME) and 33 normal stromal (HMS) cell lines were established using an organoid isolation method after digestion with enzymes for 24 hours. The HME cell lines were characterized by immunohistochemistry (IHC). Ploidy was determined by karyotype and Interphase FISH mapping with centromere probes for Chromosomes X and 17. Cellular morphology was observed both on two-dimensional and in three-dimensional culture systems. The HME cell lines were subjected to FACS analysis using multiple antibodies including CD24, CD44, Muc1, CD49f, and EpCAM.

**Results:** 96.9% of early passage cells are diploid. The HME cells express vimentin, CK 5/6, p63, CD 10, CK 18, and HER-1 when tested on a set of 145 normal colon and colon cancer samples from patients (GSE8671). This model perfectly predicted no Wnt activity on a public set of 32 normal colon samples plus 32 colon adenomas from patients (GSE20916). The model again predicted no Wnt activity in all 44 normal samples, and an active Wnt pathway in all cancer samples, except for four of the 36 samples from surgically removed colon carcinomas, which most likely are the most heterogeneous.

Thirdly, we took the latter model, trained on colon samples, and tested it on three breast cancer data sets, one with 51 breast cancer cell lines (GSE12777) and two sets of breast cancer samples from patients (GSE12276, n = 204; GSE21653, n = 266). Also on this different tissue type, our model correctly predicted Wnt activity for the few breast cancer cell lines that are known to have an active Wnt pathway. For the two patient studies, a significantly higher number of samples of the basal subtype were predicted to have an active Wnt pathway compared to the other subtypes (Fisher’s exact test: p = 0.021 for GSE12276; p = 2.7e-5 for GSE21653), in line with increasing evidence for Wnt activation in the basal subtype.
Conclusions: Phenotypic plasticity is common to all the HME cell lines characterized to date. Differentiation into cells of mesodermal and ectodermal origin, CD49+/EpCAM- by FACS, and the presence of multiple nucleoli suggest that the isolated cells are a multipotent/ stem cell residing in the normal adult breast. These cells, through a series of yet to be elucidated events, may be the cells of origin of MCB.

P5-05-03
The Effect of Breastfeeding on Molecular Characteristics of Invasive Breast Cancer.
Ellsworth RE, Valente AL, Kane JL, Shriver CD. Henry M. Jackson Foundation, Windber, PA; Windber Research Institute, Windber, PA; Walter Reed Army Medical Center, Washington, DC

INTRODUCTION: Breastfeeding has been associated with an overall decreased risk of developing breast cancer and the protective effect of breastfeeding has been associated with decreased risk of triple negative and estrogen-responsive breast cancer. Although the mechanism by which breastfeeding provides a protective effect is not well-defined, physical changes in the mammary epithelium reflecting maximal differentiation, may be involved.

METHODS: The database of the Clinical Breast Care Project was queried to identify all patients born between 1946 and 1964 (“baby boomers”) with invasive breast cancer who had given birth to at least one living child. Clinicopathologic characteristics were compared between patients who breastfed for at least six cumulative months and those who never breastfed using chi-square and Fisher’s exact tests. RNA was isolated after laser-microdissection of 24 pairs of breastfeeding and non-breastfeeding tumors matched by stage, subtype and grade. Gene expression data was generated using HG U133A 2.0 microarrays and analyzed using the Partek Genomics Suite using a FDR<0.05, 2-fold expression difference to define significance.

RESULTS: Of the 1,008 parous women with invasive breast cancer in the database, 325 (33%) breastfed at least 6 months, 165 (16%) breastfed less than 6 months, and 518 (51%) never breastfed. Women who breastfed ≥6 months were not included in additional analyses. Age at menarche and first birth and number of children did not differ between groups; age at diagnosis was significantly (P<0.0001) lower (54.5 years) in women who breastfed compared to those who did not (61.0 years). No differences were detected between patients who breastfed and those who did not for ethnicity or for any tumor characteristics, including tumor stage, grade or size, hormone, HER2 or lymph node status, or subtype. At the molecular level, however, 21 probes representing 19 genes were differentially expressed between women who breastfed and those who did not. Eleven genes, including HAPLN1, had significantly higher and 8 genes, including LGALS7, had significantly lower expression in tumors from women who did not breastfeed.

CONCLUSIONS: Despite the matching of tumor samples, and lack of differences in pathological characteristics between women who breastfed ≥6 months and those who did not breastfeed, differentially expressed genes were identified between tumors from the two groups. Many of the differentially expressed genes have been associated with prognosis, especially in ER negative breast tumors. These data suggest that breastfeeding does alter the underlying molecular characteristics of invasive breast tumors, and may reflect alterations in exposure to estrogen levels during breastfeeding.

P5-05-04
Bisphenol-A Modulates Estrogen Receptor Alpha and Compromises Tamoxifen-Induced Apoptosis.
Goodson III WH, Luciani MG, Sayeed A, Jaffee I, Moore II DH, Dairkee SH. California Pacific Medical Center Research Institute, San Francisco, CA; Kimmel Cancer Center, San Francisco, CA; California Pacific Medical Center, San Francisco, CA; University of California, San Francisco, CA

BACKGROUND: Antiestrogens are important for treatment and prevention of breast cancer. Such drugs influence multiple pathways involved in regulation of cell survival - including apoptosis. In previous work, exposure of human, benign, high-risk donor breast epithelial cells (HRBECs) to the xenoestrogen (XE) bisphenol-A (BPA) induced gene expression changes predicting disruption of apoptotic signaling (Dairkee et al, Cancer Research 2008). Therefore, we hypothesized that antiestrogenic, pro-apoptotic effects of tamoxifen are likely modulated under the influence of XEs. Because the environmental XE , BPA is routinely detected in a large percentage of random human blood samples, we employed functional tests to determine whether BPA interferes with tamoxifen-induced apoptosis in XE-exposed HRBECs.

METHODS: With IRB approval and written informed consent, HRBECs were obtained by random periareolar fine needle aspiration (RPFNA) from women who were high risk on the basis of personal or family history of breast cancer, breast density, or high-risk histology. Primary HRBEC cultures were treated for one week with a low concentrations of BPA corresponding to levels reported in human body fluids. Estrogen Receptor (ER)-α and -β were measured by Western blot (n=6). Altered response to tamoxifen toxicity was measured by analyzing quantitative changes in the Annexin V-positive apoptotic cell population (n=8).

RESULTS: After one-week exposure to BPA, ER-α increased in six of six cases, ER-β decreased in 5 of 6 cases, and we observed a 25 percent reduction in tamoxifen-induced apoptosis. Prior exposure to BPA at the high concentration range detected in the human population reduced tamoxifen efficacy by 50 percent. There was a dose response of evasion of apoptosis to increasing concentrations of BPA (P<0.001, two-sided test for slope fixed effect in a linear mixed effects regression). Prior exposure to another XE, methylparaben (MP), caused a similar dose-dependent decline in tamoxifen-induced apoptosis (p<0.001).

CONCLUSION: BPA and MP, in a range of concentrations measured in humans, prevent tamoxifen-induced apoptotic cell death. For BPA this involves upregulation of ER-α and downregulation of ER-β. Because these XEs are widespread in the American population, they may play a role in limiting the prophylactic and therapeutic efficacy of tamoxifen and other hormone-based therapies.

P5-05-05
Human Papilloma Virus Identification in Intraductal Papilloma and Breast Cancer Using Broad-Spectrum Primers.
Balci FL, Bender O, Coskun F, Duzgan AP, Saylam B, Yuncu E, Rota S, Fidan I, Feldman S, Soran A. Numune Training and Research Hospital, Ankara, Turkey; Okmeydani Training and Research Hospital, Istanbul, Turkey; Gazi University School of Medicine, Turkey; Columbia University College of Physicians and Surgeons, New York; University of Pittsburgh, Magee-Womens Hospital, Pittsburgh

BACKGROUND: There is limited data about Human Papilloma Virus (HPV) presence in intraductal papilloma, and there are some conflicted studies about the effects of HPVs on breast cancer. Most
of these studies have methodological pitfalls as well. We conducted a retrospective paraffin block study to understand the presence of HPV in papilloma and cancer tissues with broad-spectrum primers. **Methods:** Twenty seven intraductal papilloma and 18 breast cancer patients' paraffin blocks were obtained from the patients who underwent ductoscopy aided papillomectomy or mastectomy. In totally 87 histopathological samples, HPV-DNA were identified by real time PCR and broad-spectrum genotyping was performed by in situ hybridization. The cancer patients were evaluated according to the status of Estrogen Receptor (ER), Progesteron Receptor (PR) and ErbB-2 positivity. **Results:** The mean age±SD was 49±16 in papilloma and 52±14 in breast cancer patients. We found that 29.6% (8 of 27) of intraductal papillomas and 44.4% (8 of 18) of breast cancer paraffin blocks had HPV-DNA positivity by PCR in situ hybridization with broad spectrum primers. Fifty percent (4 of 8) of HPV-DNA positive papillomas had only type 11; in one, only type 6; in one, mixture of type 6,11; in one, mixture of type 11, 39; and in one, mixture of type 11, 39, 82 was detected. Breast cancer lesions had only type 11 of HPV-DNA positivity in 62.5% (5 of 8) of cases; in one, mixture of type11, 39; in one, mixture of type 11, 82; in one, mixture of type 6, 11, 39 was detected. In seven HPV-DNA positive cancer patients, ER /PR and/or ErbB-2 was positive, but it was only positive in one triple negative patient. **Conclusion:** HPVs may have a role in developing papilloma and breast cancer. Validation of HPVs role in the etiology of breast cancer/ papilloma, require further studies to understand whether the HPVs are episomal or integrated during the tumorigenesis.

**P5-05-06**  
Roe T, Hoar F, Tzelepis C. University of Birmingham, Birmingham, United Kingdom; City Hospital, Birmingham, United Kingdom  
**Background:** Mounting evidence implicates iron in the development of a number of cancers, including breast. The responsible mechanism is poorly understood in breast cancer, although recent work has demonstrated irregular expression of various molecular iron transporters.  
**Materials and Methods:** Immunohistochemistry on archived material was used to evaluate differences in expression of iron and haem exporters, importers and storage proteins between breast cancer, DCIS and normal breast tissue. The results were corroborated by Western blotting and RT-qPCR on protein and RNA samples generated from matched normal and cancer specimens collected at mastectomy. The ability of benign and malignant breast cells in vitro to accumulate excess iron in the presence of exogenous iron/ haem was evaluated with a ferrozine assay. In addition the effect of exogenous iron and haem on cell phenotype in vitro was assessed in benign and malignant cell lines. Assays utilised included viability (MTT), proliferation (BrdU), migration, anchorage-independent growth (colony formation) and invasive capacity. These assays were then repeated in the presence of iron chelators to investigate their potential utility as a therapeutic modality.  
**Results:** Statistically significant increases (p<0.05) were observed in expression of the iron importers DCytB, DMT1 and TFR in breast cancer relative to normal tissue. Levels of the storage protein ferritin were also significantly raised. The iron exporters ferroportin and hephaestin were significantly under-expressed in breast cancer, while hepcidin (which induces exporter degradation) was over-expressed. Levels of the haem importer HCP1 and the exporters BCRP and FLVCR were all significantly down-regulated in cancer relative to normal. Both benign and malignant breast cells were capable of importing excess iron, although only malignant cells could import excess haem. Iron induced increased viability and proliferation in benign cells but had no effect on their migratory or invasive capability, or their ability to form colonies. Haem had no effect on benign cell phenotype. Iron and haem both had significantly positive effects on all these aspects of cell phenotype when introduced to malignant breast cells in vitro. Iron chelators were capable of abrogating these positive effects.  
**Discussion:** Archived material and prospectively collected matched tissue pairs demonstrate that breast cancers exhibit altered expression of the proteins involved in transporting iron and haem through the cell. In vitro experiments reveal malignant cells to be capable of taking up both substances, which both appear to drive a more malignant phenotype. These in vitro changes can be reversed through the introduction of iron chelators, implying possible efficacy as a novel therapy in breast cancer.

**P5-05-07**  
The Msp1 Polymorphism of Cytochrome P-450 CYP1A1 in Asymptomatic Women with Breast Cysts.  
Fenile R, Nazário A, Facina G. Federal University of São Paulo, São Paulo, SP, Brazil; Federal University of São Paulo, Brazil; Federal University of Sao Paulo, Brazil  
The objective of this study was to determine the prevalence of the cystic form of fibrocystic breast changes among women from different age groups correlated with the presence of the Msp1 polymorphism of cytochrome P-450 CYP1A1. **Methods:** A retrospective, case-control study (was carried out with ultrasound examination) was performed on 197 women divided into two groups: 44 women with cystic breast disease (cases) and 153 women without breast disease (controls). The Msp1 polymorphism of CYP1A1 was detected by PCR and DNA sequencing. The Msp1 polymorphism was defined as the presence of the Msp1 allele within the CYP1A1 gene. **Results:** The mean age±SD was 49±16 in papilloma and 52±14 in breast cancer patients. We found that 29.6% (8 of 27) of intraductal papillomas and 44.4% (8 of 18) of breast cancer paraffin blocks had HPV-DNA positivity by PCR in situ hybridization with broad spectrum primers. Fifty percent (4 of 8) of HPV-DNA positive papillomas had only type 11; in one, only type 6; in one, mixture of type 6,11; in one, mixture of type 11, 39; and in one, mixture of type 11, 39, 82 was detected. Breast cancer lesions had only type 11 of HPV-DNA positivity in 62.5% (5 of 8) of cases; in one, mixture of type11, 39; in one, mixture of type 11, 82; in one, mixture of type 6, 11, 39 was detected. In seven HPV-DNA positive cancer patients, ER /PR and/or ErbB-2 was positive, but it was only positive in one triple negative patient. **Conclusion:** HPVs may have a role in developing papilloma and breast cancer. Validation of HPVs role in the etiology of breast cancer/ papilloma, require further studies to understand whether the HPVs are episomal or integrated during the tumorigenesis.

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**Discussion:** Archived material and prospectively collected matched tissue pairs demonstrate that breast cancers exhibit altered expression of the proteins involved in transporting iron and haem through the cell. In vitro experiments reveal malignant cells to be capable of taking up both substances, which both appear to drive a more malignant phenotype. These in vitro changes can be reversed through the introduction of iron chelators, implying possible efficacy as a novel therapy in breast cancer.
P5-06-01
Gene Expression Analysis of Resistance to Bevacizumab in a VEGF-Reinforced Xenograft Model of ER-Positive Breast Cancer.
Gökmen-Polar Y, Toroni RA, Goswami C, Sanders KL, Mehta R, Sirimalle U, Tanasa B, Shen C, Li L, Ivan M, Badve S, Sledge GW. Indiana University School of Medicine, Indianapolis, IN; University of Medicine and Pharmac, La Jolla, CA

Background: Bevacizumab, a monoclonal antibody targeting vascular endothelial growth factor (VEGF), had promising therapeutic efficacy in breast cancer. However, intrinsic or acquired resistance is common in the clinic. To improve our understanding of the underlying mechanisms of resistance to bevacizumab (BEV), we report the gene expression analysis of resistance to bevacizumab in a VEGF-overexpressing xenograft model of ER-positive breast cancer.

Methods: We developed a nude mouse xenograft model of resistance to anti-VEGF therapy with BEV in which MCF-7 control (ML20) or MCF-7 VEGF (MV165) transfectants were implanted in mammary fat pads, allowed to grow, then treated with BEV, with collection of tumor at early or late time points (while responding (R) to or progressing (NR) on anti-VEGF therapy). To elucidate differentially expressed gene profiles associated with tumor resistance to BEV, we performed whole-genome gene expression analysis (Human WG-6v2 Expression Beadchips, Illumina) and miRNA profiling (TaqMan ArrayHuman MicroRNA A+B Cards Set v3.0, Applied Biosystems). Validation of the chosen genes was performed using quantitative real-time RT-PCR (qRT-PCR).

Results: Gene expression analysis revealed differentially regulated genes in the MV165-NR group compared with the MV165-R group. Among the significant genes, Follistatin (FST) and HEY2 were the top genes upregulated in NR compared to R by ANOVA. Expression of HEY2 is induced by the Notch signaling pathway. Using qRT-PCR, we validated the expression of FST and Notch in our system. FST1 was significantly decreased (Fold change= -3.2; P=0.03) in the R group compared with vehicle in MV165 xenografts. In contrast to R group, FST was upregulated significantly (Fold change= 9.3; P=0.05) in the NR group. Notch4 displayed increased levels of expression in NR group, but it did not reach significance (P=0.23). In addition, correlation of mRNA and miRNA profiles showed that miRNAs targeting FST and Notch4 were differentially regulated in NR group compared to R group in MV165 xenograft tumors. Among the miRNAs, TGF-β-induced oncomiR miR-181a is up-regulated in NR and targets both FST and Notch4. Other miRNAs that target both Notch4 and FST include miR-1, miR-133a, miR-133b, and mir-449b.

Conclusion: Our data serve as a potential mechanistic explanation for acquired resistance to bevacizumab. These data may shed light on the transitory effect of BEV observed in the E2100 first-line metastatic breast cancer trial, where VEGF-targeted therapy prolongs progression-free survival in metastatic breast cancer without improving overall survival.

P5-06-02
Response and Acquired Resistance of BRCA1-Deficient Triple-Negative Breast Cancer Xenografts to Alkylators or PARP Inhibitors.
Sirimalle U, Tanasa B, Shen C, Li L, Ivan M, Badve S, Sledge GW, Jonkers J. Netherlands Cancer Institute, Amsterdam, Netherlands; Bellvitge Biomedical Research Institute (IDIBELL), Barcelona, Spain; Gene function, Breakthrough breast cancer research centre, London, UK

Background. BRCA1 mutated breast tumor cells are defective in DNA repair by homologous recombination and therefore especially sensitive to treatment with DNA double-strand break (DSB) inducing agents, such as alkylators or PARP inhibitors. However, such tumors can eventually develop therapy resistance. Understanding therapy resistance mechanisms may help in designing treatment strategies to overcome therapy resistance.

Methods. We have developed BRCA1-deficient triple-negative breast cancer (TNBC) xenograft models by implantating fresh human breast tumor pieces and subsequent serial passaging. These models show epigenetic loss of BRCA1 due to promoter hypermethylation or genetic inactivation of BRCA1 due to a frameshift mutation (c.2210delC) resulting in a premature stop codon (p.Thr737LeufsX15). We have used these BRCA1-deficient TNBC models to study response and acquisition of resistance to alkylating therapy (cisplatin, melphalan) and the clinical PARP inhibitor olaparib.

Results. Treated tumors were sensitive to the alkylators cisplatin or melphalan or the PARP inhibitor olaparib, in some cases resulting in complete remission with no palpable tumor left. However, relapses did occur in most cases and repeated treatment of recurrent tumors eventually led to acquired resistance.

Since restoration of BRCA1 function has been suggested as a mechanism of therapy resistance (Swisher et al., Cancer Res 2008; 68: 2581), we determined BRCA1 expression in therapy-sensitive and -resistant tumors by Western blot analysis. While no full length BRCA1 protein could be detected in the therapy-sensitive tumors, expression of full length BRCA1 protein was found in the majority of cisplatin resistant and olaparib resistant tumors. BRCA1 reexpression in the therapy-resistant BRCA1-c.2210delC tumors was caused by genetic restoration of the reading frame due to reversion to the wildtype sequence or additional deletions near the c.2210delC mutation. BRCA1-re-expression in the therapy-resistant TNBC xenografts with epigenetic loss of BRCA1 was caused either by loss of BRCA1 promoter methylation or by complex rearrangements at the BRCA1 locus.

Conclusion. Although BRCA1-deficient TNBC xenografts are initially very sensitive to alkylating agents and olaparib, resistance to treatment develops in almost all treated tumors. This acquired resistance is frequently associated with re-expression of BRCA1 due to secondary mutations or loss of promoter methylation.
P5-06-03
Gene Expression Associated with Breast Cancer Primary and Secondary Resistance to Neoadjuvant Chemotherapy.
Fujiki FK, Katayama MLH, Brentani H, Carraro DM, Abreu APS, Barros Filho MC, Oliveira CT, Caldeira JRF, Góes JCS, Brentani MM, Folgueira MAK. Faculdade de Medicina da Universidade de Sao Paulo, Sao Paulo, Brazil; Faculdade de Medicina da USP, Sao Paulo, Brazil; Hospital do Cancer A.C. Camargo, Sao Paulo, Brazil; Instituto Brasileiro de Controle do Cancer, Sao Paulo, Brazil; Hospital do Cancer A.C. Camargo, Sao Paulo, Brazil; Hospital Amaral Carvalho, Jau, Sao Paulo, Brazil

In breast cancer, neoadjuvant chemotherapy may provide valuable information on tumor resistance mechanisms, as variable degrees of tumor response, from pathological complete response until progressive disease, may be observed. To have a better insight of the resistance process to chemotherapy, we have compared resistant pre chemotherapy samples (representing primary or intrinsic resistance) and residual post chemotherapy from partially responsive tumors (secondary or acquired resistance), by means of their transcriptional profile. Tumors from 36 patients submitted to neoadjuvant chemotherapy (4 cycles doxorubicin and cyclophosphamide) were collected (12 pre-chemotherapy from primary resistant samples and 24 post chemotherapy from residual samples) and analyzed by cDNA microarray (training set, n=25, 9 resistant; 16 residual) and/or by qRT-PCR (technical validation set, n=11, and biological validation set, n=11; 8 resistant; 14 residual). In samples from the training set analyzed using a cDNA microarray platform with 4608 genes (Student’s t test; p <0.01; FDR <10), 138 genes were differentially expressed between resistant and residual tumors, which correctly classified tumors in unsupervised hierarchical clustering analysis, with high confidence. Genes were mainly involved in regulation of cell cycle, regulation of cell growth, cell division, protein modification and DNA dependent DNA replication. Among genes involved in cell growth and cell division, 12 were selected to be further evaluated, using qRT-PCR: MORF4L2, NOTCH2, HTRA1, DLC1, CYR61, MLH1, CHFR, CDC16, CDKL1, NOTCH2, HRAS, CDK2, in technical and biological validation sets of samples. In the technical validation group, a significant positive correlation was observed for the expression of five genes (as evaluated by microarray and qRT-PCR, Spearman correlation, p<0.05), which were then tested in discriminant analysis to detect the best combination to classify resistant and residual tumors. The best combinations included all the five selected genes, CYR61/DLC1/CDKL1/NOTCH2/HTRA1, as well as three of them, CYR61/DLC1/HTRA1, which correctly classified 100% and 91% of tumors from the technical and biological validation groups (100% and 73% in leave one out cross validation, respectively). CYR61 was more expressed in residual samples, however no other main individual gene expression differences were detected. Our data indicate that primary and secondary chemoresistance may present some differences, involving mainly cell growth and cell proliferation processes. CYR61, a CCN growth factor family member, may be synthesized by tumor cells as well as by adjacent fibroblasts, exhibiting autocrine and paracrine actions, stimulating proliferation, migration and chemoresistance. CYR61 enhanced expression in post chemotherapy samples suggests that epithelial stromal interactions may be active players in the chemoresistance process, deserving more detailed analysis for targeted therapies. Supported by FAPESP.

P5-06-04
Ekalyongco RC, Mukohara T, Kataoka Y, Kiyota N, Fujiwara Y, Minami H. Kobe University Graduate School of Medicine, Kobe, Japan; Kobe University Hospital Cancer Center, Kobe, Japan

Background: No studies have yet clarified the mechanism of acquired resistance to insulin-like growth factor-1 receptor (IGF-1R) tyrosine kinase inhibitor (TKI). Our previous study of 16 breast cancer cell lines found that only MCF-7 expressed high levels of insulin receptor substrate (IRS)-1 and was sensitive to the IGF-1R-TKI NVP-AEW541.

Material and Methods: We developed a model of acquired resistance to NVP-AEW541 by continuously exposing MCF-7 cells to NVP-AEW541, naming the model MCF-7-NR. To explore the mechanism of acquired resistance to NVP-AEW541, the effects of NVP-AEW541 on cell growth and IGF-1R signaling in MCF-7 and MCF-7-NR cells were examined.

Results: With Western blot analysis, we found that MCF-7-NR had much lower levels of IRS-1 than parental MCF-7. While phosphorylation of Akt was completely inhibited by administration of NVP-AEW541 (3 μM) in both cell lines, phosphorylation of S6K remained high only in MCF-7-NR. The notion of Akt-independent S6K phosphorylation in MCF-7-NR was further supported by the fact that cell growth and phosphorylation of S6K was affected by administration of the Akt inhibitor perifosine to a lesser degree in MCF-7-NR than in MCF-7. Further, the mTOR inhibitor everolimus inhibited phosphorylation of S6K and cell growth equally in both lines. Screening of MCF-7 and MCF-7-NR lines for phosphorylation of 42 receptor tyrosine kinases with and without 3μM NVP-AEW541 showed that Tyro3 phosphorylation remained high only in MCF-7-NR cells. Gene silencing of Tyro3 using siRNA resulted in reduced cell growth, decreased phosphorylation of phosphoinositide-dependent kinase-1 (PDK-1) and protein kinase C α/βII, reduced expression of cyclin D1 in the MCF-7-NR line, with minimal effects evident in the MCF-7 line.

Discussion: Findings from our current study support the possibility of predicting sensitivity to NVP-AEW541 by measuring IRS-1 expression, as hypothesized in our previous study. Akt-independent activation of mTOR/S6K by an as-yet-undefined mechanism appears to induce acquired resistance to NVP-AEW541, and as such mTOR inhibitors may have therapeutic value. Tyro3 upregulation and migration of control of cellular growth and cyclin D1 expression may also induce resistance to NVP-AEW541. Although the validity of these findings should be evaluated in further preclinical studies and eventually in clinical trials, our observations may lead to individualized use of NVP-AEW541 and the development of “back-up drugs” against tumors that acquire resistance to NVP-AEW541.

P5-06-05
Switching Addictions between HER2 and FGFR2 in HER2-Positive Breast Tumor Cells: FGFR2 as a Potential Target for Salvage after Lapatinib Failure.
Tsurutani J, Azuma K, Sakai K, Aro T, Nishio K, Nakagawa K, Kinki University Faculty of Medicine, Osaka, Japan; Kobe University Graduate School of Medicine, Osaka, Japan

Agents that target HER2 have improved the prognosis of patients with HER2-amplified breast cancers. However, patients who initially respond to such targeted therapy eventually develop resistance to the treatment. We have established a line of lapatinib-resistant breast cancer cells (UACC812/LR) by chronic exposure of HER2-amplified and lapatinib-sensitive UACC812 cells to the drug. The mechanism
by which UACC812/LR acquired resistance to lapatinib was explored using comprehensive gene hybridization. The FGFR2 gene in UACC812/LR was highly amplified, accompanied by overexpression of FGFR2 and reduced expression of HER2, and a cell proliferation assay showed that the IC \textsubscript{50} of PD173074, a small-molecule inhibitor of FGFR tyrosine kinase, was 10,000 times lower in UACC812/LR than in the parent cells. PD173074 decreased the phosphorylation of FGFR2 and substantially induced apoptosis in UACC812/LR, but not in the parent cells. FGFR2 appeared to be a pivotal molecule for the survival of UACC812/LR as they became independent of the HER2 pathway, suggesting that a switch of addiction from the HER2 to the FGFR2 pathway enabled cancer cells to become resistant to HER2-targeted therapy. The present study is the first to implicate FGFR in the development of resistance to lapatinib in cancer, and suggests that FGFR-targeted therapy might become a promising salvage strategy after lapatinib failure in patients with HER2-positive breast cancer.

**P5-06-06**

The RNA Binding Protein FUS Is A Potential Marker for Breast Cancer Progression and Therapy Response.

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Background: The RNA binding protein FUS is a multifunctional protein that has recently been demonstrated to be directed to target genes via non-coding RNA, where it represses transcription via disruption of the transcriptional complex. We have shown that FUS is an important regulator of prostate cancer cell growth; FUS over-expression causes G1 arrest, promotes apoptosis and blocks proliferation \textit{in vitro} and \textit{in vivo}, whereas knock-down increases proliferation. This regulation appears to be, at least in part, via direct regulation of \textit{cyclin} D1. Further, analysis of patient samples demonstrated that FUS levels are inversely correlated with Gleason grade and directly correlated with survival and the presence of bone metastases. We hypothesised that FUS is a predictive and/or prognostic marker for hormone-dependent cancers. Materials and Methods: FUS expression in breast cancer was analysed \textit{in silico} and using Western blotting and immunohistochemistry on a tumour microarray. FUS levels were knocked down by siRNA in MCF-7 cells and chemosensitivity analysed using caspase 3/7 assays.

Results: Analysis of the Finak dataset (2008) demonstrated that FUS expression is significantly lower in breast cancer compared to normal breast. Immunohistochemistry revealed that FUS expression is also inversely correlated with grade. Importantly, FUS expression was also correlated with survival (Kaplan-Meier Plotter); significantly longer relapse free survival was seen in patients whose tumours had high levels of FUS. FUS expression also appears to correlate with therapy response since analysis of the Chang dataset (2003) revealed that FUS levels are significantly lower in patients resistant to Docetaxel and we found FUS levels to be lower in the tamoxifen resistant MCF-7 cell line (MCF-7 TAMR) compared to the parental line. We therefore hypothesised that loss of FUS expression may be driving therapy failure. In support of this, reducing FUS expression in the parental MCF-7 line resulted in a significant reduction in docetaxel-induced Caspase 3/7 activity, suggesting that FUS is a regulator of chemosensitivity. Discussion: FUS may be an important regulator of breast cancer progression since its levels are inversely correlated with grade and directly correlated with relapse free survival. Further, lower FUS expression is also correlated with reduced hormone therapy and chemo-sensitivity. Since knock-down of FUS reduced MCF-7 chemosensitivity, we believe that the observed changes in FUS expression are, in part, driving therapy failure and hence propose that FUS is a novel target and biomarker for breast cancer.

**P5-06-07**

The Mechanisms of Autophagy Involved in the Effects of Everolimus (RAD001) on Overcoming Multidrug Resistance of Breast Cancer Cell Line MCF-7/ADR.

Liu Z-B, Hou YF, Xu W-H, Ling H, Shao Z-M. Breast Cancer Institute, Shanghai, China

To investigate the effects of everolimus on overcoming multidrug resistance of breast cancer cell line MCF-7/ADR and to explore its mechanisms of action. MCF-7 and MCF-7/ADR cells were treated with ADM, everolimus alone or by combination at different concentrations and different times. Changes of cell morphology were observed by microscopy. Apoptosis induced by ADM or everolimus were measured by flow cytometry with PI staining and ANNEXIN V-FITC staining. Western blot was used to evaluate the expression level of apoptosis and autophagy-related molecules. To investigate specifically the role of autophagy in everolimus treated breast cancer cell lines, we used a siRNA approach, targeting autophagy gene, ATG5. The CI for each experimental combination was calculated. Dose-dependent inhibition of cell proliferation was observed with ADM and everolimus for both 24 and 48h. MCF-7/ADR was resistant to ADM. The addition of everolimusid increased the sensitivity of MCF-7/ADR cells to ADM, as indicated by the ID \textsubscript{50} by 2.6 fold for 24h; but for 48h, everolimus is even able to reduce the ID \textsubscript{50} of ADM much higher to 5-10 times. Thus, inhibition of mTOR does appear to restore ADM sensitivity in MCF-7/ADR cells, especially for a longer synergistic time (48h). Everolimus alone induced a decrease in the phosphorylation of two downstream molecules of mTOR, including p70S6K and 4E-BP1, confirming the role of everolimus in mTOR signaling regulation, whereas everolimus in combination with ADM caused an almost complete suppression of p70S6K and 4E-BP1 phosphorylation. When cells were exposed to both everolimus and ADM for 24h, there was no loss of procaspase-3 nor was there a concentration-dependent increase in cleaved caspase protein. On the contrary, ADM administration induced the proteolytic cleavage of caspase 3, and when ADM was combined with everolimus, a further increase in the expression of cleaved caspase 3 was detected in 48h. Cells treated with CQ markedly enhanced ADM-induced cell death in MCF-7/ADR cells in early stage (24h). As observed with immunoblot analysis, cotreatment of everolimus and ADM increased expression level of ATG5 and LC3-II to -I ratios, also accelerated the rate of cell death. That’s why cotreatment with CQ resulted in a rapid drop in cell viability with complete cell death observed by CCK-8 test. Immunoblot analysis also revealed non significant change of ATG5 and LC3-II within 48h of everolimus on the base of ADM treatment. Silencing ATG5 resulted in a marked downregulation of ATG5 but increased the level of proteolytic cleavage of caspase 3 induced by ADM alone and cotreatment of everolimus and ADM when cells were exposed to both everolimus and ADM for 24h. This result was consistent with our finding using the Annexin-V apoptotic assay. For 48h, the results of proteolytic cleavage of caspase 3 were similar as when cells weren’t silenced by ATG5 siRNA. In conclusion, autophagy plays bidirectional roles in the mechanisms of tumor cells suppression of everolimus combining MCF-7/ADR. Especially in late stage, inhibiting autophagy helps to overcoming resistance of everolimus and sensitizes cancer cells to ADM-induced apoptosis.
**P5-06-08**

**Mesenchymal Stem Cells and Carcinoma-Associated Fibroblasts Sensitize Breast Cancer Cells in 3D Cultures to Kinase Inhibitors.**

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Background: Bone-derived mesenchymal stem cells (MSCs) are attracted to cancer lesions and may differentiate to CAFs. By interacting with cancer cells, MSCs and CAFs may promote cancer progression and modulate drug sensitivity.

Material and Methods: To analyze ability of MSCs and CAFs to modulate drug response, we generated spheroids of MCF-7 or MDA-MB-231 breast cancer cells in the absence or presence of human (h) MSCs or hCAFs and tested the susceptibility of the breast cancer cells to three different kinase inhibitors (TKI258, RAD001 and RAF265) as used in cancer therapy.

Results: While MSCs and CAFs did not affect the response of either breast cancer cell line to PDGFR/FGFR/VEGFR inhibitor TKI258, they sensitized breast cancer cells to the mTOR inhibitor RAD001. In MCF-7 cells, this was accompanied by increased apoptosis. hMSCs and to a lesser extent hCAFs also enhanced the cytotoxic effect of RAF inhibitor RAF265 on MDA-MB-231 cells. Searching for the mechanism that underlies the effect of stromal cells on RAF265 response we found that stromal cells inhibited RAF265-induced increase in ERK1/2 phosphorylation, supported RAF265-dependent downregulation of PKCalpha (protein kinase Calpha) and prevented RAF265-induced conversion of LC3B, a marker of autophagy. To mimic the changes in ERK1/2 phosphorylation and PKCalpha expression in response to the stromal cells, we treated cells with MEK1 inhibitor U0126 or PKCalpha inhibitor Gö6976, respectively. U0126, but not Gö6976, was as effective as hMSCs in sensitizing MDA-MB-231 cells to RAF265.

Discussion: Our data suggest that hMSCs and hCAFs increased the cytotoxic effect of RAF265 on MDA-MB-231 cells by downregulating ERK1/2 phosphorylation. In summary, this study shows that hMSCs are able to render breast cancer cells more susceptible to kinase inhibitors and that, to the most part, hCAFs to which hMSCs can differentiate are able to mimic the drug-sensitizing effects of hMSCs.

**P5-06-09**

**Acquired Sensitivity to TRAIL Mediated Apoptosis in Lapatinib Resistant SKBR3 Cells.**

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**Introduction**

Lapatinib, a dual EGFR/HER2 tyrosine kinase inhibitor is approved for the treatment of trastuzumab-refractory HER2 positive metastatic breast cancer. However, not all HER2 positive tumors respond to lapatinib and patients who initially respond frequently relapse. Understanding the molecular alterations associated with acquisition of lapatinib resistance may lead to the identification of targets to overcome resistance. Thus, the aim of this study was to examine changes in apoptosis in a model of acquired lapatinib resistance.

**Methods**

SKBR3 cells were treated with 250 nM lapatinib twice weekly for 6 months to establish the lapatinib resistant cell line SKBR3-L. The effects of TNF-related apoptosis-inducing ligand (TRAIL) and tumour necrosis factor-α (TNF-α) on cell survival and apoptosis induction were examined in SKBR3 and SKBR3-L cells, using the acid phosphatase proliferation assay and the terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL) assay. Cleavage of poly ADP ribose polymerase (PARP) was used to confirm apoptosis. TRAIL-1 and TRAIL-2 receptor expression were measured by flow cytometry (FACS).

**Results**

In SKBR3-L cells, TRAIL treatment (25 ng/ml) significantly inhibited cell survival (94.0 ± 12.0 %) compared to the SKBR3 cells (11.0 ± 2.2 %). Treatment with TNF-α (0.5 ng/ml) also resulted in significant inhibition of cell survival (68.2 ± 10.1 %) in SKBR3-L cells compared to SKBR3 cells (9.4 ± 9.0 %). TRAIL (25 ng/ml) induced a low level of apoptosis in SKBR3 cells (7.0 ± 4.0 %), whereas in SKBR3-L cells, TRAIL (25 ng/ml) induced significant apoptosis (54.8 ± 2.6 %, p < 0.001), as determined by the TUNEL assay. We confirmed by western blotting that TRAIL treatment for 72 hours resulted in higher levels of PARP cleavage in SKBR3-L cells compared to SKBR3 cells. Both western blotting and FACS analysis of TRAIL receptor expression showed that neither TRAIL-1 nor TRAIL-2 receptor expression was significantly elevated in SKBR3-L cells compared to SKBR3 cells.

**Conclusions**

SKBR3-L cells, a model of acquired lapatinib resistance, display significant sensitivity to TRAIL induced apoptosis compared to the parental cell line SKBR3. Thus, targeting TRAIL may represent a novel therapeutic strategy to overcome acquired lapatinib resistance.

**P5-06-10**

**Leptin Signaling Impacts Notch and Wnt Crosstalk in Breast Cancer.**

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**Background:** Triple negative breast cancer (TNBC: ER-, PR- and Her2/neu-) is an aggressive form of the disease that disproportionately affects women of color, has an early onset, and is associated with poor survival and a resistance to common therapeutic treatments. We have previously demonstrated an association between the adipocytokine leptin and Notch signaling pathways in breast cancer. Notch participates in a crosstalk relationship with many signaling pathways involved in carcinogenesis, including Wnt and leptin, which could in turn increase tumor burden and cell survival of MMTV-Wnt1 obese mice. We hypothesize that leptin signaling crosstalk with Notch and Wnt is instrumental in the development of drug-resistant features (increased survival and proliferation) of TNBC.

**Objective:** The aim of this study was to determine whether leptin mediated crosstalk of the Notch and Wnt pathways has a differential impact on TNBC compared to ER+ cells.

**Materials & Methods:** ER+ MCF-7 and TNBC-MDA MB-231 cells (96-well plate; 1x104 cells/well) were serum deprived for 24 hours and treated with varying doses of Doxorubicin and Cisplatin for 24h in conjunction with pharmacological doses of leptin, leptin peptide antagonist (LPrA2), Wnt agonist (Wnt-1) and antagonist (Wnt-2f-1). Cell proliferation was measured via WST assay. The effect of the various treatments on the activation of Wnt (total/p85-catenin), Notch (Notch 1-4 and JAG1/DI-4 and targets survivin/Hey2), and leptin (STAT3 and targets VEGF/VEGFR-2) signaling pathways were measured using Western blot and ELISA. β-Catenin levels were also investigated by IHC in DMBA-breast cancer samples from lean and DIO (diet-induced obese mice) mice treated with LPrA2. Apoptosis was also measured.

**Results:** Leptin increased the levels of beta-catenin mainly in TNBC cells. This leptin-induced effect was also detected in breast
tumors from DIO-mice. Interestingly, leptin increased survival (bcl-2 and Caspase-3 activation) /proliferation (cell number and Cyclin D1), expression of Notch and attenuated the detrimental effects of Doxorubicin and Cisplastin on breast cancer cells. Wnt-1 had similar but less pronounced effects compared to leptin. We also observed differences in Notch expression. Moreover, MDA-MB-231 cells showed decreased response to Wnt1 in the presence of leptin.

Conclusions: Our findings suggest that leptin could play a negative role in TNBC by increasing drug-resistance through its crosstalk with Wnt and Notch signaling pathways. This may imply that obesity, characterized by elevated leptin levels, could negatively affect the outcome of TNBC treatment. Taken together, this data supports the theory that inhibition of leptin signaling could be a novel way to prevent and treat TNBC, particularly in the context of obesity and abnormal Wnt and Notch signaling. [This work was supported in part by NIH/NCI/SC1CA138658-02; NIH/ARRA/SC1CA138658-02S1 and the Georgia Cancer Coalition Distinguished Cancer Scholar Award (to RRGP); CREDO (MSCR) 2R25RR017694-06A1 (to L.S.C); N1GMS506GM08248 and NCRR 5P20RR11104 (to T.Z.M); and facilities and support services at MSM (NIH RR03034 and IC06 RR18386)].

P5-06-11 Quercetin-3-Methyl Ether Inhibits Lapatinib-Sensitive and Lapatinib-Resistant Breast Cancer Cell Growth by Inducing G2/M Arrest and Apoptosis.

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Background: Lapatinib, an oral, small-molecule, reversible inhibitor of both EGFR and HER2, is highly active in HER2 positive breast cancer as a single agent and in combination with other therapeutics. However, resistance against lapatinib is an unresolved problem in clinical oncology. Recently, interest in the use of natural compounds to prevent or treat cancers has gained increasing interest because of presumed low toxicity. Quercetin-3-methyl ether, a naturally occurring compound present in various plants, has potent anticancer activity.

Material and Methods: Quercetin-3-methyl ether was obtained from Analyticon Discovery (Potsdam, Germany). Lapatinib-resistant SK-BR-3 (SK-BR-3-Lap R) cells were isolated in the laboratory of Cell Biology and Biotherapy at the Istituto Nazionale dei Tumori, Naples, Italy. Parental SK-BR-3 and SK-BR-3-Lap R cells were cultured in monolayers at 37 °C in a 5% CO2 incubator in 10% FBS/McCoy supplemented with penicillin/streptomycin (100 units/ml; Invitrogen). SK-BR-3 Lap R cells were routinely maintained in 1 µM lapatinib. Anchorage-dependent and -independent growth and cell cycle were assessed in the presence and absence of quercetin-3-methyl ether.

Results: Here, we found that quercetin-3-methyl ether caused in a significant growth inhibition of lapatinib-sensitive and -resistant breast cancer cells. Western blot data showed that quercetin-3-methyl ether had no effect on Akt or MAPKs signaling in resistant cells. However, quercetin-3-methyl ether caused a pronounced G2/M block mainly through the Chk1-Cdc25c-cyclin B1/Cdk1 pathway in lapatinib-sensitive and -resistant cells. In contrast, lapatinib produced a pronounced G2/M block mainly through the Chk1-Cdc25c-cyclin B1/Cdk1 pathway in lapatinib-sensitive and -resistant cells. Moreover, quercetin-3-methyl ether induced significant apoptosis, accompanied with an increase in the levels of cleaved caspase 3, caspase 7 and poly(ADP-ribose) polymerase (PARP) in both cell lines.

Conclusion: Overall, these results suggested that quercetin-3-methyl ether might be a novel and promising therapeutic agent in lapatinib-sensitive or -resistant breast cancer patients.

P5-07-01 Novel Alternative Splice Variants of HER2 in Invasive Breast Cancer.

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Background: The Human Epidermal Growth Factor Receptor 2 (HER2) is an oncogene expressed in 25-30% of invasive breast cancers. HER2 shares extensive homology with other members of the HER family (HER1, HER3 and HER4), and is constitutively active as an homo- and heterodimer. The HER2 gene encodes an 185kDa transmembrane protein with tyrosine kinase activity. Gene amplification or protein expression of HER2 is a predictor of poor clinical outcome and decreased survival in women with breast cancer, and also indicates a favourable response to Trastuzumab (Herceptin) therapy, or a combinational therapy comprising Herceptin plus chemotherapy. However, resistance to Trastuzumab remains the case in approximately 50% of HER2 amplified/overexpressing tumours. Understanding the molecular mechanisms of Trastuzumab resistance and identifying more effective therapies, is critical in the treatment of patients whose breast cancers express this aggressive disease phenotype. In this study, it is postulated that the abnormal generation of mRNA splice variants may be responsible for the continued tumour growth and progression.

Aims: The aim of this study is to increase our understanding of the role of HER-2 splice variants in the development and progression of breast cancer. This will inform the development of more sophisticated and effective therapies that target specific HER-2 isoforms, rather than Herceptin, which targets just the generic wild type HER-2 protein.

Materials and Methods: The entire coding region of HER-2 cDNA was PCR amplified using 12 sets of HER-2 specific primers in HER-2 positive cell lines (SKOV-3, SKBR-3, MDA-MB-453 and MDA-MB-361).

Results: RT-PCR results showed multiple bands in various regions of the HER2 mRNA. Sequencing of these bands revealed novel alternative splice variants with deletions in exons 13 and 18 of the HER2 gene expressed in addition to the wild type HER2. Bioinformatic analysis of the deletions revealed a cassette exon deletion in exon 13, and a loss of 42 base pairs in the 3' end of exon 18 compared to the full length HER2. Both the full length HER2 sequence and the sequence containing the deletions were translated using the ExPaSy Translate Tool. This revealed an in-frame deletion of 14 amino acids and revealed a novel splice isoform with a deletion in the HER2 protein which encompasses the entire ATP binding pocket. This was determined by analysis using UniProtKB to identify the composition of amino acids for each domain of HER2.

Discussion: Our studies have identified novel splice variants in the tyrosine-kinase domain of the HER-2 gene using HER-2 positive cell lines. The loss of an ATP binding site in the HER2 gene may lead to a less active HER2 isoform which may play a significant role in prognosis. Further work is being carried out to study the regulation of these splice variants and to study the role of splice factors ASF/SF2 and SRPK1 in the regulation of HER2 splicing, and to elucidate any
significant changes in the HER2 signalling pathways. In addition, the expression of these isoforms are currently being investigated in tissues from formalin fixed, paraffin embedded and frozen breast tumours.

**P5-07-02**
**Could the Combination of COX-2 Inhibitor and Calcitriol Be a New Chemopreventive Approach To Decrease the Incidence of Breast Cancer?**
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**Background:** COX-2 is a potential molecular prognostic factor for breast cancer and 15-hydroxyprostaglandin dehydrogenase (15-PGDH) is described as a tumour suppressor in cancer. The antiproliferative effects of calcitriol (1,25(OH)2D3) mediated via the vitamin D receptor (VDR) render vitamin D a promising target in breast cancer therapy.

**Material and Methods:** The expression of the prostaglandin (PG) metabolising enzymes (COX-2, 15-PGDH), the PG receptors, the vitamin D metabolising enzymes (1-alpha-hydroxylase, 24-hydroxylase) and the VDR were determined in benign and malignant breast cell lines as well as in normal and malignant breast tissue. Measurement of PGE2 and 25(OH)2D3 serum level in healthy women and breast cancer patients was performed in winter and summertime. Additionally, the influence of calcitriol on cell proliferation was determined. In addition we examined the effect of calcitriol on the enzyme expression.

**Results:** We found an inverse correlation between the expression of the PG metabolising enzymes with the VDR as well as with the vitamin D metabolising enzymes by investigating the tissue samples. Moreover, we detected an inverse correlation between the PGE2 and 25(OH)2D3 serum level in breast cancer patients during wintertime. Furthermore the PG receptors were associated with an increased carcino genesis. The breast cell experiments presented a dysregulated vitamin D metabolism, especially in the invasive breast cell line. Calcitriol showed an antiproliferative effect only in the benign but not in the malignant cell lines, and the expression of COX-2 and 15-hydroxyprostaglandin dehydrogenase was influenced by calcitriol only in the benign breast cell line.

**Conclusions:** These results suggest a link between vitamin D and PG metabolism and therefore a possible synergism between COX-2 inhibition and calcitriol in breast cancer cells. For the first time, we could show an inverse correlation between the two metabolisms in breast cancer on different levels. Thus, the chemopreventive combination of calcitriol and/or vitamin D analogue with a COX-2 inhibitor might decrease the incidence of breast cancer.

**P5-07-03**
**Modulation of Autophagic Activity by Extracellular pH.**
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Reprogramming energy metabolism from oxidative phosphorylation to aerobic glycolysis, a common feature of human cancer, results in a relative acidic tumor microenvironment which is further accentuated by hypoxia operating within most tumors. We report that alteration of extracellular pH induces marked and rapid changes of autophagic activity in a variety of cell lines including mammary non-transformed cells and breast cancer cells. Interestingly, acidic and basic conditions induce completely opposite effect on autophagy, with its activity suppressed at lower pH whereas stimulated at higher pH. Gene knockdown experiments indicate that pH induced-autophagy requires Beclin 1, Vps34 and ATG5, key components of the autophagy pathway. Of note, acidic condition not only inhibits the basal but also blocks starvation-induced autophagy activity. Significantly, examination of different areas of tumor mass reveals a lower autophagic activity within the inner region than the outer region. These findings have important implications on the connections between autophagy and cancer as well as a wide range of other physiological and pathological processes.

**P5-07-04**
**Is α-L-Fucose Overexpressed on Cells of Aggressive Human Breast Cancers?**
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Enzymatic removal of α-L-fucose (fucose) was shown over 20 years ago to abolish metastases in vivo in a rat model of mammary adenocarcinoma [J Cell Biochem 1988; 37: 49-59]. Since that time, evidence has accumulated to indicate that fucose is a functional effector in human breast cancer; essential for construction of the invasive and metastatic phenotypes. In light of these findings, the role of fucose in aggressive breast cancers is worthy of further investigation.

Our group has modified the method of Wright et al. in order to selectively remove cell surface fucose from viable human breast cancer cells in vitro. In our experience, a 30 min incubation of breast cancer cells with α-L-fucosidase (3.2.1.51) leads to a loss of tumotransformer abilities including decreased cancer cell adhesion to select extracellular matrix components, decreased invasion into complex extracellular matrices, decreased binding by relevant antibodies and lectins, and decreased adhesion of cells (rolling) to model endothelium under shear stress flow conditions.

From this work we postulated that decisive differences in phenotypic and functional properties between treated and control human breast cancer cells should be obtainable with currently available models of aggressive disease. To that end, we and others have subsequently shown that human breast cancer cells express cell surface CD44 which carries fucosylated ligands and that its’ defucosylation altered its’ malignant phenotype. Based on these precedents, it is reasonable to hypothesize that CD44-enriched breast cancer stem cell populations require fucose in order to exert their aggressive behavior. Assays to assess the role of fucose in these stem cells are apparent and include a battery of tests involving stem cell invasion into extracellular matrices, cell binding of antibodies or fuclectins, stem cell aggregation into spheroids, colony formation in soft agar, assessment of stem cell rolling/adhesion to endothelial cells or purified E-selectin under both static or physiological flow conditions and the ability to initiate tumors upon inoculation into animal models.

We believe that new insights into aggressive breast cancer cell behaviors can be gained through the depletion of cell surface fucose from breast cancer cells. Knowledge gained from these studies should yield new insights when using current models of human breast cancer in vitro and in vivo.

**P5-07-05**
**Hypoxic Mammary Epithelial Cells in Three-Dimensional Culture Develop a Cancer-Like Phenotype.**
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Due to inadequate vascularization and tumor cell proliferation solid tumors are hypoxic compared to corresponding normal tissues. Tumor
hypoxygenation has been associated with increased metastatic potential and worse patient outcome. The hypoxic response is mainly driven by the hypoxia inducible transcription factors; HIF-1α and HIF-2α. We have reported that hypoxia leads to a less differentiated phenotype in breast cancer and neuroblastoma, and in the clinic low tumor cell differentiation is related to poor prognosis in these diseases. We have shown that high levels of HIF-2α protein correlate to distant metastasis and poor survival in invasive breast cancer. The aim of the present study was to investigate the effect of hypoxia on mammary epithelial cell differentiation and epithelial organization. We employed the well-characterized model of acinar morphogenesis on extra cellular matrix substrate. In this model mammary epithelial cells that are cultured in three-dimensional culture on a laminin-rich matrix differentiate and form acini-like structures with evacuated lumen surrounded by polarized palisade cells. We found that hypoxia (1% oxygen) impaired the process of acinar morphogenesis and the hypoxic cells failed to obtain polarity and that markers of differentiation were affected at expression levels as well as cellular localization. The hypoxic cells remained proliferative whereas normoxic cells lost Ki-67 expression within a week. We found that hypoxia impaired the global histone deacetylation that has been shown by others to take place in the process of acini formation in mammary epithelial cells. Three days post-seeding, during the proliferation stage, both normoxic and hypoxic cells stained positive for acetylated histone 4. When the normoxic cells went through morphological changes and acinar formation, the acetylation of histone 4 was reduced whereas it was maintained in the hypoxic cell-clusters at day 10 post-seeding. Thus, the hypoxic conditions maintained the epithelial cells in an acylated state, which is generally associated with an open chromatin structure, high level of transcription and an undifferentiated stage. These data indicate that hypoxia promotes loss of differentiation also at a non-malignant stage and render the cells a cancer-like phenotype. We are currently extending this study in primary cells.

**P5-07-06**

Effect of Angiotensin-(1-7) and Angiotensin II on T47D Breast Cancer Cells in the Proliferation and cAMP Production.

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Angiotensin-(1–7) [Ang(1–7)] is a peptide hormone that produces opposite responses to those of the well-characterized peptide angiotensin II (Ang II). The main actions of Ang(1–7) and AngII are mediated by MAS and Angiotensin type 1 receptor (AT1R), respectively. Ang(1–7) has blood pressure and proliferative effects contrary to those of AngII, exhibits significant angiogenic activity and may be a novel therapeutic agent for lung cancer treatment targeting a specific AT(1-7) receptor (Soto-Pantoja et al., 2009). Here, our aim was to evaluate T47D breast cancer cell proliferation and cAMP production after treatment with Ang (1–7) and AngII. By means of the cyclic AMP (cAMP) competitive enzymeimmunoassay system we measured cAMP content of T47D cells. Cell proliferation was measured using incorporation of BrdU after Ang (1–7) and AngII. In this model mammary epithelial cells that are cultured in threedimensional culture on a laminin-rich matrix differentiate and form acini-like structures with evacuated lumen surrounded by polarized palisade cells. We found that hypoxia (1% oxygen) impaired the process of acinar morphogenesis and the hypoxic cells failed to obtain polarity and that markers of differentiation were affected at expression levels as well as cellular localization. The hypoxic cells remained proliferative whereas normoxic cells lost Ki-67 expression within a week. We found that hypoxia impaired the global histone deacetylation that has been shown by others to take place in the process of acini formation in mammary epithelial cells. Three days post-seeding, during the proliferation stage, both normoxic and hypoxic cells stained positive for acetylated histone 4. When the normoxic cells went through morphological changes and acinar formation, the acetylation of histone 4 was reduced whereas it was maintained in the hypoxic cell-clusters at day 10 post-seeding. Thus, the hypoxic conditions maintained the epithelial cells in an acylated state, which is generally associated with an open chromatin structure, high level of transcription and an undifferentiated stage. These data indicate that hypoxia promotes loss of differentiation also at a non-malignant stage and render the cells a cancer-like phenotype. We are currently extending this study in primary cells.

**P5-07-07**

Follistatin Suppresses In Vitro Growth of Breast Cancer Cells and Its Reduced Expression in Breast Cancer Associated with Poor Differentiation and Prognosis.

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Background: Bone morphogenetic protein (BMP) belongs to TGF-β superfamily, being widely involved in prenatal and postnatal development and homeostasis of various tissues and organs. BMP signaling has been implicated in tumorigenesis and disease progression of certain malignancies. Recent studies demonstrated a profound role played Noggin, a BMP antagonist in bone metastasis from breast cancer. Current study examined expression of Follistatin, another BMP antagonist in a breast cancer cohort, and its relevance to pathological and clinical parameters.

Methods: Expression of Follistatin was examined in breast cancer tissues (n=93) and normal background tissues (n=30) using quantitative real time PCR and immunohistochemistry (IHC). The breast tissue samples were collected immediately after surgery. The clinical follow-up was routinely performed after surgery. The median follow-up period was 120 months (June 2004). Full-length human Follistatin coding sequence was cloned into a plasmid vector. Following transfection and verification, effect of Follistatin overexpression on cell growth was examined using an in vitro growth assay.

Results: IHC revealed cytoplasmic staining of Follistatin in mammary epithelial cells in breast tissues. The staining appeared to be weaker in breast cancer, but there was no significant difference in its transcripts levels compared with that of normal background tissues. Follistatin transcripts were decreased in both moderately differentiated and poorly differentiated tumors, p=0.0165 and p=0.0169 in comparison with well differentiated tumors respectively. Transcript levels of Follistatin in primary tumors tended to be lower in the patients with poor prognosis (33.8±14.7) including those patients had local recurrence, metastases and died of breast cancer, p=0.09 when compared with that of patients remained disease free (17.0±46.0). Its expression appeared particularly lower in the patients with breast cancer. Current study examined expression of Follistatin in primary tumors and metastases of breast cancer. Overexpression of Follistatin exerted inhibitory effect on in vitro growth of MDA-MB-231 cells.

Conclusions: Decreased expression of Follistatin in primary tumors of breast cancer correlates with poorer differentiation and mortality. Follistatin suppresses in vitro growth of breast cancer cells. It suggests that Follistatin is a negative regulator for tumor growth and disease progression of breast cancer.
P5-08-01
Quantitative Analysis of Strain Ratio of Microcalcification Lesions with Surgical Specimen Mammography.
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Measurement of the strain induced by compression can be useful in the diagnosis of breast tumors. Microcalcification (MC) detected by mammography could be the only symptom of early breast cancers. The purpose of this study was to develop a quantitative method for a differential diagnosis of MC lesions based on the strain ratio (SR) obtained by applying different breast compression pressure during mammography.

Methods: This prospective study was conducted with institutional review board approval and the written informed consent was obtained. Twenty-five mastectomy specimens (24 for treatment of breast cancer and 1 from contralateral prophylactic mastectomy) with MCs were examined by digital mammography. There were 24 malignant MCs (mean size of 18.8 mm) detected in the 24 separate specimens, including 14 lesions of invasive carcinoma and 10 lesions of carcinoma in situ. Five benign MCs (mean size of 21.6 mm) were detected, including four lesions which coexisted with malignant MCs in the four separate specimens, and one was discovered in the prophylactic mastectomy specimen. Each specimen experienced four different magnitudes of compression force and the corresponding images were acquired for further analysis. Images in Digital Imaging and Communications in Medicine format (DICOM) were exported into custom software, written in Matlab, to contour the region of interest. Stress was defined as the force divided by the whole specimen area, and the square root of MC area was calculated to represent strain deformation. The SR was the slope of the best-fit line through the four points of each lesion. Histology of the MC was correlated with SR. Receiver operating characteristic (ROC) curve analysis was performed to evaluate the diagnostic performance of SR for discrimination between benign and malignant MC.

Results: The SRs were higher in the malignant MC group (139.10 a.u.) than in the benign MC group (34.09 a.u.; p = 0.024). The SR between invasive carcinoma and carcinoma in situ showed no statistical significance (127.03 a.u. vs. 156.00 a.u.; p = 0.642). The area under the ROC curve for the diagnosis of benign and malignant MC was 0.825 (95% CI, 0.667 to 0.982).

Conclusion: This study demonstrated that the SR detected by mammography showed potential for distinguishing benign from malignant MCs. Further in vivo investigations of larger cohorts are necessary to validate these results.

P5-08-02
Mammographic Breast Texture Predicts Benign Biopsy Results and Composition.
Duewer FW, Kerlikowske K, Tlsty T, Fan B, Parvin B, Ma L, Shepherd J. UCSF, San Francisco, CA; LBNL, Berkeley, CA

Purpose: Investigate the relationship between pathology diagnosis, biopsy composition, and breast texture measured in digital mammogram in women who have had previous benign findings in their breast. The long-term goal of this project is to determine whether or not global breast texture is associated with tissue composition and biopsy results. Such a finding would be of interest because it would provide evidence that mammography images contain information related to breast biology beyond average density.

Materials and Methods: A total of 124 women who had previously received benign biopsies underwent an additional dual-energy/low dose full-field digital mammography (DXA) scan of the unaffected breast. Three measures of breast density (percent dense area (PD), percent fibroglandular volume (%FGV), absolute fibroglandular volume (FGV)) from mammogram, 15 biopsy types including apocrine metaplasia and non-secretory papillary adenoma, and four tissue compositions (collagen percent area, fat percent area, ductal percent area, epithelial percent area) were estimated from biopsies for each participant. In addition 45 breast texture features were measured on each screening image taken during the dual-energy mammography using custom software. Tissue composition at the biopsy location was estimated based on the average percent area of stained collagen, fat, ductal, and epithelial from up to 3 slides taken from the biopsy.

Results: Neither mammographic percent density, %FGV, nor FGV showed significant association with either biopsy type or tissue composition. However, some of breast texture features were significantly associated with tissue type and tissue composition. The strongest associations were found between collagen percent area and the neighborhood gray tone difference matrix texture strength (R=-0.26, P=0.005) and ductal percent area and the gray level contrast matrix homogeneity (GLCM HOM) (R=-0.30, P=0.001).

Conclusion: This study demonstrated that the SR detected by mammography showed potential for distinguishing benign from malignant MCs. Further in vivo investigations of larger cohorts are necessary to validate these results.

P5-08-03
How Reader’s Training, Software, and Image Formats Impact Percent Dense Area Measures.
Fan B, Duewer F, Wu FF, Kerlikowske K, Vachon C, Shepherd JA. University of California San Francisco, San Francisco, CA; Mayo Clinic College of Medicine, Rochester, MN

BACKGROUND: Mammographic percent dense area, the percent ratio of dense to total breast area in a mammogram, is one of the strongest measures of a woman’s risk of breast cancer. However, systematic differences have been observed between readers and mammography technologies (film and digital) that could cause clinically inconsistent associations with risk. The purpose of this study was to evaluate inter- and intra-reproducibility of percent dense area between readers and between film and digital technologies.

METHODS: One hundred digitized film mammograms were randomly selected with 25 films in each of quartile of percent density and read by two readers at two different sites (Mayo Clinic and UCSF). The readers had extensive experience and were also jointly trained at university of Toronto using Cumulus software. After training, all films were read twice with at least one year between duplicate readings. The Mayo clinic reading used Cumulus while UCSF used custom semiautomatic software to estimate total and dense tissue area. In addition, digitized films and unprocessed full field digital mammograms of the same women were assessed by one reader.
reader. The time between the film and digital acquisitions ranged from nine to twenty-four months. Interclass correlation coefficient (ICC) was calculated for each comparison.

RESULTS: The intra- and inter-observer ICCs, consistency for film images, were 0.96 (UCSF) and 0.97 (Mayo), and 0.96 (UCSF vs. Mayo). We found ICC between film and digital mammograms for percent dense area was 0.88. The digital mammogram had 9% significantly higher total breast area and 5% significantly lower percent density area compared to film.

CONCLUSIONS: Similarly trained readers had a high reproducibility regardless of the software used. Our results suggest centralized reader training should enable pooling of film breast density results from different clinics. However, pooling film and digital results would need careful calibration due to lower measured percent dense areas than on film.

P5-08-04
Mammographic Microcalcifications and Breast Cancer Tumorigenesis: A Radiologic-Pathologic Analysis.
Naseem M, Murray J, Hilton JF, Han D, Hogevsein S, Heersink RL, Muradali D, Simmons C, Bell D, Haq R, Brezden-Masley C. St. Michael’s Hospital, Toronto, Canada; University of Toronto, Toronto, Canada

Background: Microcalcifications (MCs) are tiny deposits of calcium in breast soft tissue. They serve as key diagnostic radiological features for localization of malignancy. Approximately 30% of early invasive breast cancers have fine, granular MCs detectable on mammography; however, their role in breast cancer tumorigenesis is currently unknown. The purpose of this study was to investigate the relationship between mammographic MCs and breast cancer pathology.

Methods: A retrospective chart review was performed for 882 women treated for breast cancer between 2000-2010 at St. Michael’s Hospital. Demographic information (age and menopausal status), tumor pathology (size, histology, grade, nodal status and lymphovascular invasion), hormonal status (ER and PR), HER-2 overexpression and presence of MCs were collected for breast cancer patients. Chi-square tests were performed for categorical variables and t-tests were performed for continuous variables. All tests were two-sided and p-values less than 0.05 were considered statistically significant.

Results: A total of 826 patient charts were included; 56 (6.4%) patients had metastatic carcinoma and were excluded from analysis. Only 37.0% (326/882) of the patients presented with mammographic MCs. Patients were more likely to have MCs if they were HER-2 positive (51%) as opposed to being HER-2 negative (33.4%) (p=0.001). There was a significant association between MCs and being perimenopausal with a mean age of 50 (65.2%) (p=0.012). Patients with invasive ductal carcinomas (39.7%) were more likely to present with MCs than were patients with other tumor histology (p=0.001). There was a positive correlation between MCs and tumor grade (p=0.051), with grade III tumors (41.8%) presenting with the most MCs, followed by grade II (37.9%) and grade I (29.8%). There was no significant association between mean age, mean tumor size, ER and PR status with the presence of MCs.

Conclusion: This is the largest study that suggests the appearance of MCs on mammograms is strongly associated with HER-2 overexpression, invasive ductal carcinoma and perimenopausal status. Since HER-2 is implicated in mediating aggressive tumor growth and metastasis, future studies should investigate the molecular pathways underlying HER-2 overexpression and MC development. This would help better understand the role of MCs in breast cancer tumorigenesis.

P5-08-05
Withdrawn by Author

P5-08-06
Comparison of Mammographic Density between Ductal Carcinoma In Situ and Benign Breast Disease.
Lee JW, Shin EJ, Yi OV, Lee JW, Shin HJ. Asan Medical Center, Seoul, Korea

Background: High breast density is an independent risk factor for breast cancer as well as benign breast diseases. However there are few studies about breast density of ductal carcinoma in situ (DCIS) and benign breast disease (BBD). In this study, we investigated patterns of breast density of DCIS and BBD, and comparatively analyzed breast densities of DCIS and BBD.

Material and Methods
From 2008 to 2009, 345 patients underwent surgery for DCIS at Asan Medical Center, and 295 patients for BBD at 2010. We retrospectively reviewed each groups and estimated cranio-caudal view of digital mammogram of unaffected breast by computer-assisted thresholding methods, using Cumulus 4, version 4.0. For BBD we included atypical ductal hyperplasia, fibroadenoma, papilloma(atypical/intraductal), phyllodes tumor(benign/borderline), and excluded unavailable mammogram(mostly, because of scanned films from outside hospitals and previously diagnosed breast cancer).

Results
Mean ages of each group were 46.51 year old in DCIS group and 41.43 year old in BBD group, and the mean percentage density(PD) were 44.89% and 45.27%, respectively.

Mean PD of the BBD group and the DCIS group

<table>
<thead>
<tr>
<th>Disease entity</th>
<th>Age</th>
<th>Mean PD(%)</th>
<th>SD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BBD group</td>
<td>295</td>
<td>44.27</td>
<td>11.26</td>
<td>0.79</td>
</tr>
<tr>
<td>DCIS group</td>
<td>295</td>
<td>44.89</td>
<td>18.84</td>
<td>0.18</td>
</tr>
</tbody>
</table>

There was no significant difference between two groups in total population (p=0.79). We categorized into two groups according to the age, ≥50 and <50, for each DCIS and BBD group. Mean percentage density of below 50 year old was 52.92% in DCIS group and 49.58% in BBD group (p=0.031). Mean percentage density above 50 year old was 33.67% in DCIS group and 33.29% in BBD group (p=0.867).

Mean PD of each group in the subgroup aged under 50 and over 50

<table>
<thead>
<tr>
<th>Disease entity</th>
<th>Age</th>
<th>Mean PD(%)</th>
<th>SD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign breast diseases</td>
<td>&lt;50</td>
<td>49.58</td>
<td>15.54</td>
<td>0.011</td>
</tr>
<tr>
<td>DCIS</td>
<td>≥50</td>
<td>52.92</td>
<td>16.05</td>
<td>0.20</td>
</tr>
<tr>
<td>Benign breast diseases</td>
<td>≥50</td>
<td>33.29</td>
<td>16.20</td>
<td>0.869</td>
</tr>
<tr>
<td>DCIS</td>
<td>≥50</td>
<td>35.67</td>
<td>16.62</td>
<td>0.53</td>
</tr>
</tbody>
</table>

Conclusion
The mean values and distribution patterns of PD were similar between BBD group and DCIS group. However, in the subgroup aged under 50, the DCIS group has significantly higher breast density than the BBD group. Although the causal association of breast density with breast cancer has been relatively well documented, this kind of cross-sectional study has an inherent limitation. The absolute difference between the DCIS group and the BBD group under age 50 (3.34% in table2) needs more investigation for its clinical implications.

P5-09-01
Breast Cancer Annual Screening Program Núcleo Mama Porto Alegre (NMPOA): Imaging Results after Seven Years.
Caleffi M, Amaral C, Zignani J, Grauendz M, Duarte Filho D. Hospital Moinhos de Vento, Porto Alegre, Rio Grande do Sul, Brazil

Introduction: The NMPOA cohort started in 2004 with the purpose of testing a breast screening model for underserved women in limited resource countries. In the city of Porto Alegre (South Brazil with
1.5 million population), breast cancer incidence rate is 127/100.000 (Brazilian NCI, 2010) with increasing mortality due to 45% of Stage III and IV and no public health care early detection program, it was mandatory to test the feasibility of an organized screening program for women with very low income and level of education (64% under 6 years of schooling). In this scenario, there was always a concern of over diagnosis that will increase costs (more biopsies and surgeries) and under diagnosis by the radiologists involved in the program. We present here the results of all breast biopsies performed in this period, and its correlation with the attributed BI-RADS classification in order to optimize care and costs. Also, this study has a long term proposal and other results have been published concerned to the baseline profile of the population (Cancer Epidemiol Biomarkers Prev. 2010 Oct;19(10):2673-9. Epub 2010 Aug 17).

Methods: Since April 2004, 5592 women from 40 to 69 years were enrolled in the program and underwent to clinical breast exam and annual mammography screening at NMPOA. Initially, lesions were classified as B1, B2 or B0. After complementary views or exams, B0 was classified as B3, B4 (A, B or C) or B5. B4 and B5 were considered positive tests and immediately submitted to biopsy (open surgical or ultrasound guided core biopsy). B3 was followed according to recommended in BI-RADS fourth edition, and subsequently classified as benign (annual follow up) or suspicious findings (biopsy performed). The evaluation included histological analysis of breast biopsy specimens by two independent pathologists. Predictive positive value (PPV), predictive negative value (PNV) and accuracy (Acc) were calculated.

Results: A total of 259 breast lesions were initially classified as BI-RADS 0 (recall rate of 2.8%), and further classification of these was: 166 B3 (64.1%), 60 B4 (23.2%) and 33 B5 (12.7%). Thirty seven B3 (14.3%) were considered suspicious after 6 months and submitted to biopsy. Correlation results of the 130 biopsies performed are summarized on [Table1]. PPV of 63.4% (52.8%-73.1%), PNV of 97.3% (85.8%-99.9%) and an Acc of 73.1% were observed. In this sample, 71.7% of BC were ductal invasive carcinoma, 11.7% ductal carcinoma in situ, 5% lobular invasive carcinoma and 11.6% were other types of neoplasia.

Conclusion: Recall rate is in accordance with the medical audit benchmarks recommended of less than 10% published in BI-RADS fourth edition. NMPOA is model for limited resource countries in breast cancer imaging diagnosis. The radiologists involved in the program are classifying breast lesions in accordance with the established parameters, with a high accuracy in diagnosis. Even though we are dealing with a poorly educated cohort not used to any form of screening, our adherence is 57% in 12 months and 71% in 24 months.

P5-09-02
Reducing Excess Biopsies: Improving Screening through Risk Stratification and New Thresholds for Intervention.
Kim DN, Kim J-H, Flowers CI, Elias S, Moore DH, Esserman LJ. Athena Breast Health Network, San Francisco, CA; Moffitt Cancer Center, Tampa, FL; University Medical Center Utrecht, Utrecht, Netherlands; University of California, San Francisco, CA

Background
BI-RADS Category 4 patients have a 2-95% risk for malignancy and are generally recommended for breast biopsy with little discrimination for risk level or distinction between risk of invasive or in situ disease. Our study sought to determine if higher thresholds for biopsy based on stratifying for risk and distinguishing between risk of invasive cancer and DCIS could reduce biopsy rates and increase cancer-to-biopsy yields without missing cancers urgent for resolution.

Methods
108 BI-RADS 4 cases with final outcomes data were evaluated from a prospective cohort of 215 consecutive patients seen at a same-day multidisciplinary breast clinic for women with mammograms categorized as BI-RADS 0, 4, or 5 in 2006-07. Final outcomes were determined from pathologic diagnosis or two-year follow-up. Risk estimates (RE) for DCIS and invasive cancer were collected prospectively and re-assessed by a radiologist blinded to outcomes and prior reading assessments. Cases were stratified according to the risk ranges of the BI-RADS 4 subcategories and risk of invasive or in situ disease. Biopsy rates, cancer-to-biopsy yields, and number of malignancies missed were calculated for various thresholds for intervention.

Results
A ROC curve for invasive cancer risk for the radiologist demonstrated a 98.5% level of accuracy (95% confidence interval [CI]: 96.9%, 100%). 60 cases had some risk for invasive cancer and 48 had some risk for DCIS. There were 14 invasive cancer and 11 DCIS outcomes, of which were high-grade. Pathologic assessment from biopsy or surgery was available for 100 patients. The outcomes of 8 cases were determined by benign two-year follow-up.

There are several strategies for intervention that improve biopsy yield and reduce biopsies for benign disease as shown in Table 1.

If cases with RE between 2-10% for DCIS or invasive cancer were not biopsied, 23% of biopsies would be avoided and the yield would increase to 30%. If cases with invasive cancer RE between 10-95% and DCIS RE between 50-95% were biopsied, 52% of biopsies would be avoided and the yield would increase to 39%. One invasive ductal carcinoma (3 mm, Grade 2) would be missed, although with six-month follow-up, this would not be a problem.

Limitations
Small sample size; one radiologist providing RE may not be representative of general mammographic assessment.

Conclusion
Setting higher biopsy thresholds for BI-RADS 4 lesions can safely reduce biopsy rates and increase biopsy yields. Given evidence suggesting that low/intermediate grade DCIS may be overdiagnosed, distinguishing between DCIS and invasive cancer risk at screening by offering active surveillance as an alternative to biopsy for BI-RADS 4 lesions suspicious for non-high-grade DCIS may be a promising approach for reducing biopsies. This will be prospectively tested in a reader study using several radiology readers in a series of 750 cases in the Athena Breast Health Network. New biopsy thresholds can be set if the results of our study can be validated.
P5-09-03
Health Literacy Affects Use of Screening Mammography in an Underinsured Population.
Komenaka IK, Nodora J, Hsu C-H, Machado L, Klemens AE, Zenuk R, Bouton ME, Martinez ME, Weiss BD. Maricopa Medical Center, Phoenix, AZ; University of Arizona, Tucson, AZ

Background: In late 2009 significant controversy arose when some screening recommendations were changed to advocate screening mammography starting at age 50 rather than the long standing recommendation of starting at age 40 years. Few would argue, by contrast, that patient compliance with screening mammography, starting at either 40 years or 50 years, is at optimal levels. National data indicate that many more lives would be saved by improving compliance with screening recommendations in individuals ≥50 than would be saved with screening individuals age 40-50 years. The current study was performed to determine if health literacy was associated with use of screening mammography in an underinsured population.

Methods: Maricopa Medical Center is the county safety net hospital in Phoenix, Arizona. 944 patients were seen at the Breast Clinic from January 2010 to January 2011. 638 were at least 40 years old and therefore candidates for screening mammography. Sociodemographic variables were collected. Use of mammography was asked of patients and checked by medical records. Health literacy was assessed using the Newest Vital Sign (NVS) validated screening instrument, which categorizes health literacy on 6-point scale as low health literacy likely (0-1 point), low health literacy possible (2-3 points), or health literacy adequate (4-6 points). Differences in patient characteristics were evaluated based on a Fisher’s exact test for categorical variables and one-way ANOVA for continuous variables. Multivariate analysis was then performed to determine which patient factors were associated with use of screening mammography.

Results: Among women 40 years or older, only 35% used routine screening mammography. For women 50 years or older, 38% underwent screening mammography. Among women 40 years or older and those 50 years or older, significantly more with health literacy adequate (NVS 4-6) obtained screening mammography than did women in the two lower groups (NVS 0-1 and 2-3). In groups (40+: 65% vs. 30% and 30%, p = 0.001, 50+: 65% vs. 33% and 37%, p = 0.001). Multivariate analyses adjusted for insurance status, employment, white race, Hispanic ethnicity, marital status, language, use of alcohol, family history, NVS and education level demonstrated that patients with adequate health literacy were more likely to use screening mammography (OR 3.66; 95% CI 2.14 – 6.27; p < 0.01) than patients in the two lower literacy groups. Similarly, uninsured patients were significantly less likely to undergo screening mammography (OR 0.57; 95% CI 0.38 – 0.86; p = 0.01) than those with insurance. Patients with adequate health literacy and insurance were (OR 8.61) more likely to use screening mammography than patients who were uninsured and were in the two low literacy groups. Patient race, ethnicity, language, employment, income, education level, and family history of breast cancer were not associated with use of screening mammography in this underinsured, undereducated population.

Conclusions: Use of screening mammography was poor in this underinsured population. Limited health literacy and lack of insurance are risk factors for failure to obtain mammography. Interventions to increase use of screening mammography among uninsured patients with limited health literacy are needed.

P5-09-04
Predictors of Malignancy and Surgical Outcomes Following Indeterminate Core Needle Biopsy in the British Breast Screening Programme.
Gillespie HS, Lowry K, Somerville J, McIntosh SA. University of Aberdeen., Foresterhill, Aberdeen, Aberdeenshire, Scotland, United Kingdom; Belfast City Hospital, Belfast, County Down, United Kingdom

Background: The National Health Service Breast Screening Programme (NHSBSP) invites women between 50 and 70 years for mammography every three years, with abnormal imaging leading to further assessment. Core needle biopsy (CNB) is the standard for obtaining a definitive pre-operative diagnosis. However a proportion of CNBs will be reported as showing indeterminate pathology, with uncertain malignant potential. This necessitates surgical diagnostic excision biopsy, with attendant potential morbidity, and highlights the importance of pre-operative diagnosis. This study aimed to categorise the histological lesions prompting B3 classification arising within a screening programme, and to quantify the definitive surgical outcomes following excision biopsy.

Material and Methods: Data was collected retrospectively from January 2000 – December 2010, including demographic details, radiological presentation, relevant clinical findings, results of pre-operative investigations, reports of diagnostic excision biopsy and subsequent surgical management. Patients were included if they had a B3 CNB result and subsequent excision biopsy, following attendance for screening mammography. Primary outcome was classification of B3 lesions and positive predictive values (PPV) for malignancy. Secondary outcomes were subsequent surgical procedures.

Results: There were 239 B3 lesions, representing 7.1% of all CNB carried out within the screening programme. Mean patient age was 55.6 years, and median lesion size was 14.5 mm (range: 4-25mm). Eighty seven lesions (36.4%) were malignant on excision: 28 (11.7%) invasive and 52 (21.8%) in situ. Lesion specific PPVs for malignancy were as follows: phylloides tumour 50.0%, atypical ductal hyperplasia 40.0%, columnar cell lesion 40.0%, papillary lesion 26.7%, lobular neoplasia in situ 25.7%, and radial scar/complex sclerosing lesions (RS/CSL) 25.9%. The PPV of RS/CSL with atypia was 65.2%, and without 25.8%. Of 87 malignant cases, 55 (63.2%) went on to have a further surgery. 27 (50.9%) had re-excision of surgical margins, 10 (18.2%) had a mastectomy, 8 (14.5%) had a wide local excision, 2 had an axillary biopsy and one patient had a quadrantectomy. 18 patients had two further operations, the second was most commonly an axillary clearance, and 3 patients had three further operations. Discussion: B3 lesions are heterogeneous in nature, and continue to mandate surgical excision, demonstrated by a PPV of 36.4%, the highest reported figure in the literature to date. B3 CNB represents a significant surgical workload, demonstrated by the fact that 63.2% cases go on to have further surgery, and that 8.8% will require more than one further operation. Currently there is no pre-operative investigation that reliably predicts the likelihood of B3 associated malignancy. It has been demonstrated that the use of large volume vacuum assisted biopsy can reduce the rate of under diagnosis of both in situ and invasive malignancy, and this would clearly reduce the requirement for repeated surgery reported in this study.
P5-09-05
Non-Palpable Lesions Findings in a Breast Cancer Opportunistic Screening in South Brazil.
Rockenbach R, Rombaldi RL, Caleffi M. Hospital L Geral, Caxias do Sul, Rio Grande do Sul, Brazil; Hospital Moinhos de Vento, Porto Alegre, Rio Grande do Sul, Brazil

Introduction: Today breast cancer is a major public health challenge in developing countries with increasing mortality rates due to the lack of early detection programs and access to care. Mammography has been considered the most sensitive test for breast non-palpable lesions. The Brazilian National policy recommends mammography from age 50 and every two years. This study investigates only radiological findings in a set of women seeking for long term breast care surveillance. Objectives: To evaluate patients characteristics of cases with non-palpable breast lesions.

Material and method: A prospective and transversal study was undertaken between March, 2009 and April, 2010 on 110 women with non-palpable breast lesions, diagnosed by mammography or ultrasound. Histo-pathological evaluation was performed in core biopsy specimen. The following variables were studied: pregnancy, parity, menarche, menopause, hormone replacement therapy, breast lesion location, tumor size, histopathology findings, mammographic and ultrasound classification – BI-Rads.

Results: 23.7% (n=26/110) out of 110 women were diagnosed with breast cancer and 76.3% (n=84/110) were diagnosed with benign mammary pathology. Mammography, according BI-Rads classification, strongly suggested (p<0.000) the presence of benign and malignant pathologies. In the breast cancer group, 14 were diagnosed with stage I (TNM), compared to the official data from INCA where stage I is 10% of the cases.

Mean 52±10.7 49.5±2.8 0.153
> 60 years 5 0.150
50-59 years 9 25.7 26 31.1
30-39 years 2 7.7 14 16.7
Age range of non-palpable breast lesions (n=110)

Conclusion: Mammographic screening reduces mortality by 30% after 50 years of age and also provides important benefit (20%) in younger women (30-49). Our study showed a cancer detection rate of 25.7% and 34.6%, respectively, demonstrating the importance of screening also in younger ages. Overall, the rate of biopsy positive was very satisfactory (1 out of 4 cases). Among the studied non-palpable breast lesions, our findings resulted in 53.8% stage I breast cancers (TNM) which reinforces the urgent need of a structured screening program in Brazil to save more lives.

Financial support: Avon Institute.

P5-10-01
Metabolic Pharmacodynamic Effect Evidenced by 18FDG-PET as a Tool for Early Prediction of Iniparib Efficacy in Metastatic Triple Negative Breast Cancer (MTNBC): A Proof of Concept Study.
Kerrou K, Gligorov J. Eastern Paris University Hospitals, APHP Tenon, Paris, France; Eastern Paris University Hospitals, APHP Tenon - APREC, Paris, France

Rationale:
It was hypothesised that Iniparib or 4-iodo-3-nitrobenzamide acts as an irreversible inhibitor of PARP1 and possibly other enzymes. It is shown to maximally promising then confusing results in MTNBC in combination with chemotherapy. To date, its mechanism of action has not been completely elucidated but since 1992, inhibition of glycolysis by iniparib and subsequent necrotic cell death has been described. We hypothesised that early inhibition of glycolysis evidenced by in-vivo imaging with 2-((18)F)Fluoro-2-Deoxy-D-Glucose Positron-Emission Tomography (18FDG-PET) may be a predictor of efficacy in iniparib, as it was previously reported for other anticancer drugs.

Objectives:
To detect metabolic pharmacodynamic effect of iniparib plus chemotherapy by means of 18FDG PET and proof the concept of its use as an early predictive tool of Iniparib efficacy in MTNBC.

Patients & Methods:
From March to November 2011, 8 pts were included in our institution in a randomized phase II study comparing bi-weekly versus weekly iniparib treatment in combination with gemcitabine and carboplatin. All patients had 18FDG-PET at baseline, at day 7 (D7). The maximum value of standardized uptake value (SUVmax) of all measurable metastatic lesions was measured and the median SUVmax was calculated for all patients at baseline and D7. The metabolic response was defined as the percentage of reduction of SUVmax (SUVmax Baseline – SUVmax D7) / SUVmax Baseline and the median SUVmax response was correlated to RECIST 1.1 response at six weeks and to disease-free survival (DFS).

Results:
The median decrease in SUVmax of evaluable lesions measured at D7 was predictive of RECIST 1.1 response at six weeks and was correlated to the best obtained response during the treatment phase. Furthermore, it showed a linear correlation with DFS (y = -0.0007x - 0.1325; R² = 0.3402).

Conclusion:
Metabolic pharmacodynamic effect as evidenced by 18FDG PET as soon as one week of treatment act as an early predictive tool of Iniparib plus chemotherapy efficacy in MTNBC. Considering the lack of a predictive biomarker of response and if confirmed on larger study, 18FDG PET could be used as a surrogate predictive biomarker in this setting. These results may be considered as an in-vivo proof of concept for the inhibition of glycolysis by iniparib.

P5-10-02
Relevance of Magnetic Resonance Imaging To Predict Disease-Free Survival after Neoadjuvant Chemotherapy of Breast Cancer.
Loo CE, Vrancken Peeters M-JTFD, Wesseling J, Gilhuijs KG. NKI-AVL (Netherlands Cancer Institute - Antoni van Leeuwenhoek Hospital), Amsterdam, Netherlands; University Medical Centre Utrecht, Utrecht, Netherlands; NKI-AVL, Amsterdam, Netherlands

Purpose:
To explore the relevance of magnetic resonance imaging (MRI) to predict disease free survival (DFS) after neoadjuvant chemotherapy (NAC) of breast cancer.

Patients and Methods:
Between July 2000 and December 2007, MRI examinations were performed in 188 women before, during and after NAC in the context of a single-institution review board approved study. Patients were consecutively included after informed consent. Interpretation of MRI
was revised by an experienced breast MR radiologists and included assessment of lesion morphology, changes in morphology, size, and contrast uptake kinetics (initial and late enhancement).

MRI features and known prognostic markers of breast cancer (age, tumor response at pathology, tumor size at baseline, immunohistochemistry-derived breast cancer subtype (triplet-negative; HER2-positive or ER-positive/HER2-negative)) were correlated with DFS. Multivariate Cox regression analysis was used to explore the impact of relevant associations.

**Results:**

Follow-up data were available for 184 women. The median follow-up time was 57.6 months (range 32.4 - 125). In 30 women events were found (26 breast cancer related deaths; 29 distant metastasis and 12 local/regional recurrences).

At multivariate analysis, only breast cancer subtype (p=0.004) and largest diameter of late enhancement at MRI after NAC (p=0.006) retained prognostic value in favour of response at final pathology, age and tumor size at baseline. Cumulative survival at 60 months (kaplan-meyer curves) was 70%, 86.7%, and 89.7% for triple-negatives, HER2-positive and ER-positive/HER2-negative subtypes [figure 1], respectively, and 88.5%, 81.4 % and 55.1% for absence of washout/plateau after NAC, between 0 and 30 mm washout/plateau, and ≥ 30 mm washout/plateau, respectively [figure 2].

**Conclusions:**

MRI after NAC in conjunction with breast cancer subtype has potential as complementary predictor for DFS. Larger diameters of residual late enhancement at MRI after NAC are associated with inferior DFS at 60 months.

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**P5-11-01**

**The Accuracy of Preoperative Ultrasonography Guided Vacuum-Assisted Breast Biopsy in Determining Histological Type, ER Status, PgR Status, HER2 Status and Ki67 Level in Invasive Breast Cancer.**

**Fujita T, Sawaki M, Hattori M, Kondo N, Horio A, Ushio A, Gondo N, Adachi E, Iwata H. Aichi Cancer Center Hospital, Nagoya, Japan**

**Background:** Recently, ultrasonography guided vacuum-assisted breast biopsy (US-guided VABB) has been widely used as alternative to surgical open biopsy. Enough breast tissue samples obtained by US-guided VABB are important because of increasing neoadjuvant treatment. Although the information obtained from US-guided VABB may be the only information available for determining the candidates for neoadjuvant treatment, only few studies evaluated the concordance of histological type, estrogen receptor (ER) status, progesterone receptor (PgR) status, human epidermal growth factor-2 (HER2) status, and Ki67 level between US-guided VABB and surgical specimen. The aim of this study was to evaluate the accuracy of preoperative US-guided VABB.

**Materials and Methods:** In 439 breast cancer patients without neoadjuvant treatment who underwent US-guided VABB and surgical resection from April 2004 and March 2011 at Aichi Cancer Center hospital, we examined the concordance of Histological type, ER status, PgR status, HER2 status, and Ki67 level between US-guided VABB and surgical specimen. All the US-guided VABB were performed using 11-gauge Mammotome® or 10-gauge VACORA®. The ER and PgR status were assessed using Allred scoring system by IHC. These statuses were categorized as positive when the total score was more than two. HER2 expression status was tested by IHC and FISH. HER2 3+ by IHC, or 2+ and FISH positive were judged as HER2 positive. In this study, the Ki67 cut-off level for positivity was defined at 20% (Penaulet-Llorca et al, JCO 2009).

The agreement on histological type, ER status, PgR status, HER2 status, and Ki67 level were tested using the absolute concordance rate and the kappa statistic values.

**Results:** The concordance rate of histological types between US-guided VABB and surgical specimens was 93.4% (410 of 439 cases) with a Kappa statistic value of 0.82. In 115 cases diagnosed as DCIS by US-guided VABB, 28 cases (24.3%) were subsequently diagnosed as invasive cancer by surgical specimens. However, among these cases, 78.6% (22/28) were T1mic and T1a. And one case (0.3%, 1/324) diagnosed as invasive cancer by US-guided VABB changed DCIS by surgical specimens. The concordance rate of ER, PgR, and HER2 status were 96.6% (112/116), 89.5% (102/116), and 97.4%(113/116), respectively (kappa statistic value of 0.99, 0.76, and 0.90). In HER2 status, the concordance rate between US-guided VABB and surgical specimens was better than between core needle biopsy and surgical specimens (the concordance rate: 88%, kappa statistic value: 0.65, Usami et al, Jpn J Clin Oncol 2007). The agreement of Ki67 level was 85.7% (24/28) with a Kappa statistic value of 0.71.

**Conclusions:** The judgment of histological type, ER status, and HER2 status by preoperative US-guided VABB can be used with confidence to determine the treatment strategies according to molecular subtype.

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**P5-11-02**

**Prediction of Results of MammaPrint’s 70 Gene Signatures by Conventional Histopathological and Biological Approaches in Patients with Breast Cancer.**


**Background:** Several studies have shown that MammaPrint’s gene signatures allow prognostic stratification of breast cancers that is superior to the currently used clinico-pathological risk factors. MammaPrint has also been reported to predict chemotherapy response. The aim of this study was to find out whether conventional histopathological and biological approaches can be used to predict MammaPrint results.

**Materials and Methods:** 96 invasive breast carcinomas were studied (of them, 34 cases with positive lymph nodes). For MammaPrint, fresh material from breast carcinomas was sent to Agendia (Amsterdam) in the provided RNA-laters. Conventional assays included: (i) histopathologic parameters combined according to a so called “Graz Risk Assessment” scheme (“GRA”, see below), (ii) immunohistochemistry for MIB-1 (Ki-67) as the “only parameter”, (iii) Nottingham Prognostic Index (NPI), (iv) uPA/PAI-1 (Elisa, cut-off for uPA: 3 ng/mg, cut-off for PAI-1: 14 ng/mg), and (v) flow cytometry (either >6% or >10% for SPF). The “GRA” consisted of 4 “main” factors (high nuclear grade, MIB-1 >20%, Her2/neu pos. (immunohistochemistry or FISH), vascular invasion (either lymphatic or blood vessels) or lymph node metastasis) and 5 “minor” factors (ER neg., PR neg., p53 >10%, tumor necrosis and age > 40 y.o.). Tumors were classified as high risk when at least 4 parameters or 2 “main” factors were present. For MIB-1 scoring, at least 500 cancer cells were counted in each case. Different MIB-1 cut-offs (10%, 15%, 20%, 25%, and 30% or more) were tried. All analyses were performed independently and the results of each method were blinded. Overall accuracy, sensitivity, specificity, positive predictive value (PPV),
P5-11-03
Real-Time Imaging of Human Breast Tissue with Reflectance Confocal Microscopy: Correlation with Routine Pathology.

Fogarty SP, Shiffert MT, Berezowski K, Hartmann D, Cabrera MC, Sidawy MK, Furth PA, Liu MC. Georgetown University, Washington, DC

Background: Near-infrared reflectance confocal microscopy (RCM) allows for immediate noninvasive 3-D optical sectioning of opaque objects, such as human tissue, without using the potentially destructive staining and fixing methods used with routine pathology. Recently, RCM has been used to differentiate between malignant and non-malignant dermatologic conditions. We hypothesize that this technique can be used to efficiently and reliably evaluate human breast tissue for the presence of malignancy without compromising the ability to perform routine immunohistochemical (IHC) analyses that accompany the diagnosis of invasive breast cancer.

Methods: 45 core needle breast biopsies (12mmx2mm) were collected under sonographic guidance. Biopsy specimens were immediately placed in phosphate buffered saline, injected with 5% acetic acid to enhance reflectivity of the nuclei, and imaged within 5-10 minutes. Digital images of the nuclear and cellular morphology from each intact specimen were acquired and catalogued within 1 hour of biopsy using the VivaCell 5000. Tissue samples were then formalin-fixed and sectioned for routine H&E evaluation or IHC assays. A board certified pathologist trained on 16 paired RCM images and H&E slides was given the blinded test set created from these 45 breast biopsies and asked to evaluate the RCM images for the presence of carcinoma. Preliminary evaluation was also done on 5 biopsy samples with known estrogen receptor (ER) and progesterone receptor (PR) status to determine the feasibility of assessing ER/PR on tissue treated with acetic acid for RCM.

Results: Routine H&E staining identified invasive carcinoma 10/45 biopsy samples (9 invasive ductal [IDC], 1 invasive lobular [ILC]); 2 specimens with IDC also contained ductal carcinoma in situ (DCIS). Evaluation of the RCM images led to the same diagnosis of invasive carcinoma vs. not in all 45 samples and correctly identified 6/9 IDC and 1/1 ILC. RCM correctly identified 1/2 specimens known to contain DCIS. RCM misclassified 3 IDC as DCIS (1), ILC (1), or lobular carcinoma in situ (1), and 1 DCIS as ILC. With respect to the determination of ER/PR status, RCM accurately assessed positivity for both receptors in all 5 specimens.

Conclusions: RCM is comparable to standard microscopy for the reliable identification of carcinoma, and the ability to evaluate breast tissue for malignancy using this technology allows for real-time pathology and may negate the need for repeat diagnostic biopsies to ensure adequate sampling for diagnosis. Importantly, the ability to perform routine IHC for ER/PR status after tissue processing for RCM appears to be preserved. Further evaluation applying the existing technology to a larger sample size for histopathologic correlation, assessment of ER/PR status, and evaluation for HER2 status are planned. These encouraging findings support interest in tailoring RCM for breast tissue to improve the ability to distinguish between invasive vs. in situ disease, and ductal vs. lobular histology.

P5-11-04
A New Immunohistochemistry-Based Assay SBrC5 Classifies Invasive Breast Cancer Subtypes – Profiling Five Biomarkers in One Single Test.

Liu H, Muralitharan S. Thermo Fisher Scientific, Fremont, CA

Background: Obtaining expression status of estrogen receptor (ER), progesterone receptor (PR) and Her2 is essential for guiding treatment and predicting outcome for breast cancers. By adding cytokeratin 5/6 and EGFR to the above mentioned three common biomarkers, a five-biomarker breast cancer panel has been validated in previous studies for identifying the basal-like subtype with superior prognostic values than the triple negative subtype1. The current study aimed to evaluate a new immunohistochemical assay, named Subtyping Breast Cancers 5-in-1 Assay (abbreviated as SBrC5, or “the assay” in this poster), for classifying breast cancer subtypes.

Material and Methods: A cocktail of ER, PR, Her2, cytokeratin 5/6 and EGFR antibodies (SBrC5 Cocktail) and the MultiVision Detection Kit (TFS Cat. #: TL-012-MARH) were used in generating the data for this poster. The Multivision Detection Kit is a double staining system with two visualizing colors, red and blue. The assay was performed on a breast cancer tissue microarray containing 75 cases in duplicate. Both color and cellular localization of the staining generated from the assay was used for the result interpretation. There were three staining clusters: 1) Red Nuclei indicated ER or PR positive; 2) Red Membrane indicated Her2 expression; and 3) Blue Membrane or Cytoplasm indicated EGFR or cytokeratine 5/6 positivity. The concordance for the assay when compared to the above mentioned three common biomarkers, and predicting outcome for breast cancers. By adding cytokeratin 5/6 and EGFR to the above mentioned three common biomarkers, a five-biomarker breast cancer panel has been validated in previous studies for identifying the basal-like subtype with superior prognostic values than the triple negative subtype1. The current study aimed to evaluate a new immunohistochemical assay, named Subtyping Breast Cancers 5-in-1 Assay (abbreviated as SBrC5, or “the assay” in this poster), for classifying breast cancer subtypes.

Results: Significant association with MammaPrint on univariate analysis for both receptors in all 45 specimens and correctly identified 6/9 IDC and 1/1 ILC. RCM correctly identified 1/2 specimens known to contain DCIS. RCM misclassified 3 IDC as DCIS (1), ILC (1), or lobular carcinoma in situ (1), and 1 DCIS as ILC. With respect to the determination of ER/PR status, RCM accurately assessed positivity for both receptors in all 5 specimens.

Conclusions: RCM is comparable to standard microscopy for the reliable identification of carcinoma, and the ability to evaluate breast tissue for malignancy using this technology allows for real-time pathology and may negate the need for repeat diagnostic biopsies to ensure adequate sampling for diagnosis. Importantly, the ability to perform routine IHC for ER/PR status after tissue processing for RCM appears to be preserved. Further evaluation applying the existing technology to a larger sample size for histopathologic correlation, assessment of ER/PR status, and evaluation for HER2 status are planned. These encouraging findings support interest in tailoring RCM for breast tissue to improve the ability to distinguish between invasive vs. in situ disease, and ductal vs. lobular histology.
obtain subtypes information in biopsy settings where the tumor tissue obtained is often insufficient for multiple tests required to accomplish such task by existing methods. 


P5-11-05

Garcia-Saenz JA, Romero A, Lopez Garcia-Asenjo JA, Roman JM, Moreno A, Fuentes M, Furio V, Pelayo A, Diaz-Rubio E, Caldes T, Martin M. Hospital San Carlos, Madrid, Spain; Hospital Principe de Asturias, Alcalá de Henares, Madrid, Spain; Hospital Gregorio Maranon, Madrid, Spain

Background: Measurement of residual disease following neoadjuvant chemotherapy that accurate predicts long-term survival in locally advanced breast cancer (LABC) is an essential requirement for new drugs efficacy evaluation. Several methods to assess neoadjuvant chemotherapy tumor response have been described. However, to our knowledge, agreement between methods and correlation with survival in independent prospective cohorts has not been reported.

Patients & Methods: In this study we report neoadjuvant chemotherapy tumor response and survival in 151 consecutive LABC patients, included in a neoadjuvant clinical trial (http://www.clinicaltrials.gov; NCT00123929). Patients were randomized to either neoadjuvant docetaxel 100 mg/m² every 21 days or neoadjuvant doxorubicin 75mg/m², every 21 days, for 4 cycles. Following surgery, response was established according to three methodologies: the measurement of residual breast cancer burden (RBC) as described by Symman’s (Symmans WF et al. J Clin Oncol. 2007;25:4414-22), Miller and Payne classification (Ogston KN et al. Breast. 2003;12:320-7) and RECIST criteria. Regarding to Symmans classification we have evaluate both RBC index, as a continuous variable, and RBC classes as a categorical variable (RBC-0,I,II,III). Kappa Cohen’s coefficient (K) was used to test agreement between methods. We assessed the correlation between treatment outcome and overall survival (OS) by calculating the Harrell’s C- statistic.

Results: Median of follow up was 51.9 months. All three methods showed a moderate capacity to classify patients according to OS. The C-statistic to predict OS was 0.76 (IC: 0.67- 0.84) for RBC index and 0.71 (IC: 0.64-0.78) for RECIST criteria and 0.69 (0.60-0.78) for Miller and Payne classification. Interestingly, we did not encountered any death events within RCB-0 class. No significant differences were found between C-statistic when patients were stratified according to therapy. In order to assess the agreement between techniques, we grouped categories 1 and 2 of Miller and Payne classification in 1 category. The agreement between Miller and Payne classification and Symmans method was very high (K=0.87). In contrast, we found a moderate-fair agreement between Miller and Payne classification and RECIST criteria (K=0.46) and Symmans method and RECIST criteria (K=0.27).

Conclusion: All three methods predicted fairly well OS. RCB-0 identified the best outcome group. The agreement between methodologies based in pathology analyses was very high. However, the agreement falls off when these methodologies were compared with RECIST criteria.

P5-11-06
Immunohistochemistry Discordance between Primary and Recurrent Tumors in Breast Cancer. Analysis of Potential Influence of Technique Bias by Comparing Test-Results under Two Different Conditions.


Introduction: Discordance in expression of estrogen (ER) or progesterone receptors (PgR) and HER2 has been previously described in several studies, having this discordance an important impact on therapeutic approach or even in survival. However it is unclear whether discordance is a reflex of a clonal differentiation to a more aggressive biological tumour or the consequence of technical biases or inter-explorer variability. We aimed to assess the role of technique biases in observed discordance between primary tumour (PT) and recurrent tumour (RT) by analyzing discordance in two different situations: routine versus optimal conditions.

Patients and methods: We conducted a retrospective study in patients (pts) diagnosed with a recurrent breast cancer in the Hospital Clinico of Valencia between January 2000 and June 2010. All patients had a previous histological diagnose of the PT and all underwent a second histological study of the RT. Discordance was assessed in terms of hormone-sensibility (HS) (any ER or PgR +) vs hormone-resistance (HR) (ER-/PgR -) and triple negativity (TN) (ER-, PgR-, HER2-) vs non-TN (any positive). Routine conditions were referred to the immunohistochemistry (IHC) assessment of the normal practice on the base of which therapeutic decisions were made. Routine conditions were performed in the Pathology Department of our institution but PT and RT were usually evaluated at different timing and occasionally by different pathologists. In order to assess potential laboratory biases we repeated IHC analysis under optimal conditions. For these optimal conditions analyses, paired samples from both PT and RT were re-assessed at the same time and tests were performed in the same way. Final determination of the results was made by two highly trained pathologists and in case of disagreement in any determination, both explorers re-assessed the sample at the same time.

We evaluated discordance between PT and RT under both conditions, and the accuracy of determinations in PT or RT between both conditions.

Results: 128 paired samples (from 64 different pts) were analysed. Median age of the series was 48.5 years (range 27-83). Recurrences were loco-regional in 14 pts (21.9%) and distant in 50 pts (78.1%). RT tissue was obtained from skin and soft-tissues in 18 pts (28.1%), lung in 11 (17.2%), bone in 10 (15.6%), lymph nodes in 10 (15.6%), pleura/peritoneum in 9 (14.1%) and liver in 6 (9.4%). Discordant cases between PT an RT in terms of HS/HR were 30.4% in routine conditions and 9.8% in optimal conditions. In terms of TN/non-TN were 14.3% in routine vs 8.2% in optimal conditions. Accuracy in results HS/HR between routine and optimal conditions was 80.7% in PT and 76.0% in RT. In results of TN/non-TN accuracy between both conditions was 94.2% in PT and 87.2% in RT.

Conclusions: Results suggest that an important rate of discordance observed in routine conditions might be strongly influenced by technique biases. However, changes observed in optimal conditions with 9.8% discordance in HS/HR and 8.2% in TN/non-TN suggest that in a small proportion of tumours, observed discordance might be explained by biological mechanisms.
Receptor Concordance in Triple-Negative Breast Cancer (TNBC) Recurrences.

Killian ME, De Los Santos JF, Forero-Torres A, Krontiras H. University of Alabama at Birmingham, Birmingham, AL

Background: The phenotype of TNBC is defined by absence of estrogen (ER) and progesterone receptors (PR) and lack of amplification of the HER2 oncogene. It is an aggressive form of breast cancer, and despite sensitivity to contemporary chemotherapy, TNBC have a poorer prognosis than ER positive phenotypes, marked by early relapses. This study examines the concordance of receptor status in recurrences among patients with TNBC.

Methods: After obtaining IRB approval, we identified patients with triple negative breast cancer treated at the University of Alabama at Birmingham between 1998 and 2010. Patient and tumor characteristics and disease status were recorded. Patients with ER, PR, or HER2 (3+) positivity on immunohistochemistry (IHC), or HER2 amplification by fluorescence in situ hybridization were excluded. Data regarding the receptor status of the recurrence, if known, was documented.

Results: Five hundred and two women with TNBC were diagnosed at our institution between 1998 and 2010. Of these patients, 95/502 (19%) had recurrences. Twenty-seven (28%) were local, 18 (19%) were regional, 35 (37%) were distant, and 15 (16%) were both local-regional and distant. These women ranged in age from 33 to 84 years of age. Of the women with a recurrence, 78/95 (82%) had a biopsy confirming breast cancer recurrence. Of those biopsied, 33/78 (42%) had receptor studies performed, and of these, 30 included data for estrogen, progesterone and HER2 receptors. The remaining 3 had only IHC for estrogen receptors performed. Twenty-two/thirty (73%) had concordance on their biopsy with their initial TNBC status, whereas 8/30 (27%) women developed a pathologically discordant recurrence. Conclusion: In this single institution study of TNBC patients, approximately one quarter of patients developed a pathologically discordant distant recurrence noted at biopsy. It is unclear whether patients experiencing a pathologically discordant recurrence differ in prognosis from those with concordant recurrences. Further study is necessary to evaluate this and determine whether routine biopsy based on pathologic discordance rates and outcomes are warranted.

High Concordance of 5 HER2 In Situ Hybridization Methods with Abbott FISH.

Boers JE, Netjes C, Meeuwissen HC, Prinsen C, Bart J, van der Logt EMJ, Schuuring E. Isala Klinieken, Zwolle, Netherlands; University Medical Center, Groningen, Netherlands

Background: HER2 in situ hybridization (ISH) has become a common test in breast cancer. Abbott FISH was used in most clinical studies showing the efficacy of anti-HER2 treatment in HER2 positive carcinomas. Only reports comparing one or two of newly developed ISH assays with Abbott FISH have been published previously. We conducted a comprehensive concordance study of 5 ISH methods with Abbott FISH in a large series of breast carcinomas.

Methods: Tissue Micro Arrays (TMA) were constructed by taking three 0.6 mm tissue cores from formalin-fixed/paraffin-embedded tissue-blocks from 402 primary breast carcinomas diagnosed in 2007 (supported by the Dutch Pathological Society). Up to 384 cases were analyzable in the TMA. ISH was performed after ample experience with 6 ISH assays. Scoring was performed by two independent observers without knowledge of the other ISH data according the ASCO-guidelines for HER2-testing. HER2 and chromosome 17 (Chr17) signals were counted separately, the HER2:Chr17 ratio was calculated and considered positive when the ratio was ≥ 2.0 In cases with a ratio was between 1.8 and 2.2, additional enumeration was performed. The discordant cases were reviewed and scores were reassigned on consensus of opinion. Concordance and Cohen’s kappa score were calculated.

Results: see table.

<table>
<thead>
<tr>
<th>Method</th>
<th>Analyzable cases</th>
<th>HER2 ratio ≥ 2.0</th>
<th>Concordance</th>
<th>Kappa score</th>
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<td>372</td>
<td>371 (11,6%)</td>
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<td>X</td>
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<tr>
<td>FISH, DAKO</td>
<td>353</td>
<td>366 (10,5%)</td>
<td>99%</td>
<td>0,96</td>
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<td>359</td>
<td>369 (10,3%)</td>
<td>99%</td>
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<td>dual probe SISH, Ventana</td>
<td>373</td>
<td>377 (10,2%)</td>
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<td>0,96</td>
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<tr>
<td>dualCISH, Zytovision</td>
<td>348</td>
<td>357 (10,2%)</td>
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</tbody>
</table>

High Concordance of Microarray Based Determination of ER, PR and HER2 Receptor Status and Local IHC/FISH Assessment Worldwide in 749 Patients.

Wesseling J, Cusumano G, Tinterrri C, Sapino A, Zanconati F, Lute-Holtzoi M, Nguyen B, Deck K, Querzoli P, Perin T, Giardina C, Seitz G, Guinebretiere J, Barone J, Watanabe T. Netherlands Cancer Institute, Amsterdam, Netherlands; CHC, Liege, Belgium; Istituto Clinico Humanitas, IRCCS, Rozzano, Italy; Universita di Torino, Torino, Italy; Universita di Trieste, Trieste, Italy; Medisch Spectrum Twente, Enschede, Netherlands; Locy Beach Memorial Health Care, Locy Beach, CA; Saddleback Memorial Medical Center, Laguna Hills, CA; Instituto di Patologia, Universita di Ferrara, Ferrara, Italy; Centro di Riferimento Oncologico, Aviano, Italy; Instituto di Anatomia Patologica, Univeristità degli Studi di Bari, Bari, Italy; Klinikum Bamberg, Bamberg, Germany; Centre Rene Huguenin, Saint-Cloud, France; Comprehensive Breast Care and Sharp Memorial Hospital, San Diego, CA; Hamamatsu Oncology Center, Hamamatsu, Japan

Background

The level of estrogen receptor (ER), progesterone receptor (PR) and HER2 expression is predictive for prognosis and/or treatment response in breast cancer patients. However, differences in fixation and IHC and subjective interpretation can substantially affect the accuracy and reproducibility of the results. The commercially available TargetPrint test measures the mRNA expression level of ER, PR and HER2 and provides high quality second opinion for local IHC/FISH assessment. This study compares results from the microarray-based TargetPrint with IHC and FISH (for HER2 IHC2+) generated by local standard procedures.

Methods

Prospective tumor samples were collected for 749 patients diagnosed with breast cancer stage I to IV between February 2008 and January 2011. The mRNA level of ER, PR and HER2 (TargetPrint) was assessed in the Agenda laboratories (Agenda BV, Amsterdam, the Netherlands) in fresh tumor samples submitted from 22 hospitals from Europe, New Zealand, Japan and US. The results of the IHC/FISH assessments performed according to the local standards at the hospitals were compared to the quantitative gene expression readouts.

Results

Of the 749 samples, IHC assessment was unknown for 5 ER samples and 4 PR samples; FISH was unknown for 24 samples. TargetPrint read out was not assessed for HER2 for 11 samples. Median age of these patients was 61 years. Comparison of IHC and gene expression read out by TargetPrint showed a concordance of 95% for ER; 82% for PR and 91% for HER2.
In this study, only 4% of all IHC ER positive samples were classified negative by microarray. In contrast, 14% of IHC ER negative samples were classified positive by microarray. However for HER2, 28% of IHC/FISH HER2 positive samples were classified negative by microarray and 5% of IHC/FISH HER2 negative samples were classified positive by microarray. Samples with discordant classifications for TargetPrint and local assessment are being reviewed in greater detail by a central pathologist.

Conclusions
Microarray based readout of ER, PR and HER2 status using TargetPrint is highly comparable to local IHC and FISH analysis in 749 analyzed samples in various hospitals worldwide. The results indicate mRNA expression read out for ER, PR and HER2 by TargetPrint provides high quality second opinion for local IHC/FISH assessment.

P5-11-10
Reproducibility and Robustness of the FDA Approved INFORM HER2 Dual ISH DNA Probe Cocktail Assay.


Background: Amplification and/or HER2 overexpression is associated with poor clinical outcome for patients with invasive breast carcinoma. Determination of HER2 status dictates eligibility of patients for trastuzumab (Herceptin) therapy, which has been shown to improve prognosis. We present data from the inter-observer reproducibility and robustness studies of the FDA approved INFORM HER2 Dual ISH DNA Probe Cocktail (HER2 Dual ISH) assay, which is an alternative to FISH. The assay is fully automated and is scored using light microscopy providing morphological context.

Methods: Ninety-five human invasive breast carcinoma specimens (a mix of amplified and non-amplified cases) were stained in a study, using the HER2 Dual ISH assay. The slides were enumerated by two qualified readers. The same cohort was stained using PathVysion FISH and read by one qualified reader. To verify the robustness of the HER2 Dual ISH assay, customer selectable options for factors known to impact the staining quality and intensity, which can be varied for troubleshooting, were tested on human invasive breast carcinoma cases and xenografts. The slides were evaluated by one qualified reader. For both studies all assay steps were fully automated on a VENTANA BenchMark XT automated slide stainer using silver metallographic and red colorimetric detections for Dual ISH.

Results: In the inter-observer study the first pass staining success rate was >93% for the HER2 Dual ISH assay, while the FISH first pass staining success rate was 59%. Some cases were rescued after a second attempt, which yielded a second pass staining success rate of 80% for FISH and 97% for HER2 Dual ISH. The negative, positive and overall agreement rates and 95% confidence intervals for the 61 clinical samples of this cohort that were enumerable with both FISH and the HER2 Dual ISH assay are 94.7 (75.4-99.1), 95.2 (84.2-98.7), 95.1 (86.5-98.3) and 89.5 (68.6-97.1), 97.6 (87.7-99.6), 95.1 (86.5-98.3) for reader A vs. FISH and reader B vs. FISH respectively. The positive and negative agreement rates for the inter-observer reproducibility of the HER2 Dual ISH assay were 94.7 and 97.6 %, respectively.

In robustness testing, the recommended staining protocol was shown to be effective. All conditions tested resulted in appropriate staining (except for mildest cell conditioning and protease options).

Conclusion: The fully automated FDA approved INFORM HER2 Dual ISH assay is robust, reproducible and concordant with the manual FISH assay in determining HER2 status in invasive breast carcinoma.

P5-11-11
Automated Quantification Methods Improve the Accuracy of PR as an Independent Prognostic Factor in Tamoxifen Treated Breast Cancer Patients.

Klimowicz AC, Kornaga EN, Yau A, Pohorelic BK, Petirillo SK, Konno M, Magliocco AM. Tom Baker Cancer Centre, Calgary, AB, Canada; University of Calgary, Calgary, AB, Canada

Background: Adjuvant endocrine therapy reduces the risk of recurrence and death in hormone receptor positive breast cancer patients. However, 40-50% of estrogen receptor (ER) positive tumors are resistant to endocrine therapy. We have previously shown that quantitative measurement of ER protein expression has limited value as a prognostic marker in tamoxifen treated patients. The presence of progesterone receptor (PR) expression has shown promise as prognostic and/or predictive marker for endocrine therapy, including adjuvant tamoxifen, but reports are contradictory. Automation of scoring methods will improve the accuracy of PR scoring and its value as a prognostic factor.

Methods: This retrospective study evaluated breast cancer patients treated with adjuvant tamoxifen (n=458) from 1985-2000 at the Tom Baker Cancer Centre, Calgary, Canada. Tissue microarrays were assembled from formalin fixed paraffin embedded tumor tissue. Clinico-pathologic data was obtained from chart review. Five-year disease-free survival (DFS) was the primary outcome. DAB-based PR staining was used to generate Allred PR scores, as well as to generate scores for PR percent area expression and PR integrated optical density (PR IOD) using the DAKO ACIS III scanner and image analysis software. PR tumor nuclear pixel intensity density scores were obtained using fluorescence-based PR staining, scanned and quantified with a HistoRx PM2000 scanner and AQUA image analysis software. Continuous variables were dichotimized using Xtile.

Results: In our tamoxifen cohort, 5-year DFS was associated with tumor grade [HR 4.9(3.5-6.9), p<0.001], tumor size [HR 2.7(1.9-3.8), p<0.001], lymph node status [HR 5.7(4.0-8.0), p<0.001] and ER status [HR 2.4(1.3-4.7), p=0.008]. Low PR was associated with significantly worse DFS regardless of the method used to quantify its expression. Methods of analysis requiring less subjective input had stronger associations between PR expression and outcome. The subjective identification of tumor and subjective biomarker quantification used in Allred scoring [HR 2.2(1.4-3.3), p<0.001] was less effective than ACIS scoring [PR percent area: HR 2.8(1.8-4.3), p<0.001; PR IOD: HR 2.9(1.9-4.6), p<0.001], which only requires subjective identification of the tumor. All methods were inferior to fluorescence-based AQUA scoring [HR 4.2(2.7-6.5), p<0.001], which automatically detects the tumor nuclear area using pan-cytokeratin and DAPI staining and measures the PR expression within this compartment. Multivariate analysis, which included age, tumor size, grade, tumor nuclear pixel intensity density and ER status, confirmed that AQUA [HR 3.7(2.0-5.6), p<0.001] was superior to ACIS PR percent area [HR 2.3(1.4-3.9), p=0.002] and ACIS PR IOD [HR 2.7(1.6-4.8), p<0.001], all of which were superior to Allred scoring [HR 1.2(0.7-2.1), p=0.511].

Conclusions: We conclude that: 1) PR is an independent prognostic marker in our cohort, 2) using less subjective, automated quantitative scoring methods improves the value of PR as a prognostic biomarker,
AQUA appears superior to other automated methods and that 4) the incorporation of digital image analysis into practice would improve the prognostic value of PR expression in the clinical setting.

**P5-11-12**
Correlation of Ki67 Expression between Initial Biopsy and Surgical Specimen in Untreated Breast Cancer Patients. Does Menstrual Cycle Matter?
Torrejón D, Rubio I, Sanchez-Olle G, Peg V, Sansano I, Jimenez J, Gomez P, De la Peña L, Di Cosimo S. Vall d’Hebron University Hospital, Barcelona, Spain; Solit Breast Cancer Research Group

**Background:** The measure of proliferative index by Ki67 expression is being increasingly used to identify breast cancer subtype and to guide treatment decisions. Findings on initial tumor biopsy are in some cases, such as for primary systemic treatment, the only data available prior to treatment. In addition, results from window studies to develop novel anti-tumor agents depends on initial breast cancer biopsy findings. This study aims to compare the Ki67 expression on initial biopsy with matched primary breast cancer surgical specimens. In addition, we aimed to explore the possible effect of hormonal levels - as reflected by menstrual cycle phase - on discordant Ki67 cases. Material and Methods: A total of 50 random cases were analyzed by 2 blinded pathologists. Ki67 expression was collected as a continuous variable. Tumors were then classified as high - or low-proliferative according to a Ki67 value of > or = 15% or < 15%, respectively. Spearman Rank test was used to study the correlation between initial and surgical biopsies. Data on menstrual cycle phase at the time of biopsy and surgery were retrieved from medical history of all pre-menopausal patients.

**Results:** Baseline patients characteristics are as follows: median age 48 (35-56 years), premenopausal 27 (54%), postmenopausal 23 (46%), invasive ductal carcinoma 38 (76%). A differential expression of Ki67 was found in 36 patients (72%), 20 pre- and 16 post-menopausal. Spearman’s Rank Order correlation did not show any significant difference between biopsy and matched surgical specimen in terms of Ki67 expression. Indeed, there was a strong positive correlation between initial and surgical biopsy, which was statistically significant across all patients (rs = .556, P < 0.001), and regardless the menopausal status. However, 15% of pre-menopausal patients transformed from Ki67 low to high. Therefore, data on pre-menopausal patients were analyzed according to the phase of menstrual cycle at the timing of initial and surgical biopsies. The Spearman’s Rank Order gave no correlation among 14 pre-menopausal patients with differential menstrual cycle phase at the timing of initial biopsy and surgical excision (rs = .411, P = 0.145).

**Conclusions:** Initial breast cancer biopsy can be used with high confidence for Ki67 determination. However, it should be noted that menstrual cycle phase could affect Ki67 expression as women with distinct menstrual cycle phase at the time of initial biopsy and final surgery do not show concordant results. Prospective evaluation of Ki67 expression in premenopausal patients is being planned.

**P5-11-13**
Rates of Upgrade to Malignancy for 271 Cases of Atypical Columnar Cell Hyperplasia Diagnosed by Breast Core Biopsy.
Peres A, Barranger E, Becette V, Boudinet A, Guinebretiere J-M, Cherel P. Lariboisiere Hospital, Paris; France; Institut Curie, Saint-Cloud, France

**Purpose:** Atypical columnar cell hyperplasia (ACCH) is a borderline lesion that might represent an early stage in the development of certain low-grade carcinomas in situ (CIS) and invasive cancers. There are no guidelines on its management. Our objectives were to determine the upgrade to malignancy rate and identify a subpopulation of patients that might undergo just mammographic surveillance.

**Methods:** We retrospectively reviewed the data for 271 ACCH cases among 5555 breast core biopsies obtained over a 7-year period (Jan. 2003 – Jan. 2010). We collated clinical data (age, history of cancer, menopausal status), radiological data (lesion type, size, Bi-Rads category), technical data (number of biopsies, needle gauge, excision quality) and histological data and sought correlations between these factors and upgrade rate.

**Results:** The 271 ACCH comprised 128 cases of pure ACCH, 135 cases of ACCH + atypical ductal hyperplasia, and 8 cases of ACCH + atypical lobular hyperplasia. Overall, 184 patients underwent surgery and 46 mammographic surveillance. Surgery detected 34 cases of malignancy (23 CIS, 7 invasive cancers, and 4 mixed cases) giving a 15% upgrade rate. Quality of excision was the only factor associated with under-diagnosis.

**Conclusion:** The presence of ACCH at biopsy warrants surgery.

**P5-11-14**
Flat Epithelial Atypia of the Breast: A Single Institution Experience.
Joh JE, Acs G, Kiluk JV, Laronga C, Khakpour N, Lee MC. H. Lee Moffitt Cancer Center & Research Institute, Tampa, FL

**Background:** Flat epithelial atypia of the breast is a relatively new entity of unknown significance. Our objective is to evaluate our surgical experience with this diagnosis.

**Methods:** A single institution database of breast patients from 2005-2010 was used to identify women who were diagnosed with flat epithelial atypia on core biopsy and subsequently underwent surgical excision. Patient data regarding history, type and reason for biopsy, and associated pathology was collected. Individuals diagnosed with flat epithelia atypia and cancer on core biopsies in the same breast were excluded.

**Results:** There were 52 patients who underwent surgical excision for the primary diagnosis of flat epithelial atypia. There were 3 (6%) patients with a personal history of breast cancer, 14 (27%) patients with a family history of breast cancer, and 11 (21%) patients with a concurrent new diagnosis of breast cancer in the contralateral breast. Core biopsy was recommended in most (81%) cases because of suspicious calcifications on mammography. Twenty-eight (54%) patients were found to have flat epithelial atypia associated with other atypical breast hyperplasia and 24 (46%) had flat epithelial atypia as the most significant lesion on core biopsy. In 8 (15%) patients, there was a sonographic correlate that was biopsied; 5 had only flat epithelial atypia and 3 had flat epithelial atypia associated with other atypical hyperplasia. Of the 52 patients there were 4 (8%) patients who upstaged to ductal carcinoma in-situ on surgical excision. There were no cases of invasive carcinoma. All ductal carcinoma in-situ cases were associated with other atypical breast hyperplasia, not flat epithelial atypia alone.

**Conclusion:** Though flat epithelial atypia may be associated with an increased risk of breast cancer, surgical excision of pure flat epithelial atypia may not be necessary. Larger studies are needed to corroborate these findings.
P5-11-15
Should HER-2 Score 0/1+ Breast Cancer Cases Be Retested by In-Situ Hybridisation? Results of a Multicenter Retesting Study.
Reiner-Concin AM, Lax S, Regentin P, Kronberger C, Jasarevic Z, Bogner S. Danube Hospital, Vienna, Austria; General Hospital Graz West, Graz, Austria; Medical University Graz, Graz, Austria; LKH Salzburg, Salzburg, Austria; LHK Feldkirch, Feldkirch, Austria; LKH Linz, Linz, Austria

Background: HER-2 status is vital for selection of appropriate therapy for breast cancer patients. Accuracy of immunohistochemistry (IHC) varies with a major problem of false negative testing. To assess the false negative rate retesting by in situ hybridisation was performed on a group of primary breast cancers in which according to guidelines routine retesting is not recommended.

Material and Methods: 570 breast cancers from 5 pathology departments scored 0/1+ by IHC (HercepTest or 4B5-antibody) were retested by HER2-Dual-SISH (BDISH) in a central laboratory. CAP/ASCO guidelines were applied. Cases showing ratios in the amplified or equivocal range by BDISH were further analysed by fluorescence in situ hybridisation (FISH) using Pathvysion® and ZytoLight® and retested centrally by IHC using 4B5 antibody (Ventana).

Results: 25/570 cases (4.38%) were amplified by BDISH, the majority with low level amplification (ratios ≤ 3.26). Only two cases showed high level ratios (6.35 and 6.48). 17/570 cases (2.98%) showed ratios by BDISH in the equivocal range (1.8 – 2.2). 24 amplified and 17 equivocal cases underwent further retesting. In one case no tumor tissue was available for further testing.

On further testing 17/24 (71%) BDISH-amplified cases showed high level amplification (ratios ≥ 4). Subsequently 3/13 score 2+ cases were amplified by Pathvysion® and 9/13 by ZytoLight®, 3/4 score 3+ cases were amplified by both FISH assays. In case of amplification ratios of BDISH and both FISH assays appeared to be in the same range. Only 2/17 BDISH-equivocal carcinomas were confirmed equivocal by Pathvysion® and 6/17 were confirmed equivocal by ZytoLight®. All other BDISH-equivocal cases were non-amplified by both FISH assays. Discrepant results between different methods could partly be caused by interobserver variability. This question is currently under investigation. Overall, after multistep retesting amplification occurred in only 17 cases (2.98%).

Discussion: Amplification is rare in immunohistochemically HER-2 negative breast carcinomas and occurred predominantly at low level in our study. Low level amplification seemed to be diagnosed more frequently by BDISH compared with FISH. Since clinical data demonstrate a benefit of therapy even in carcinomas with low level amplification the identification of these carcinomas might be of interest.

Conflicts of Interest: The study was supported by Roche Austria GmbH.

P5-11-16
CD44 and CD24 Expression in Ductal Invasive Breast Carcinomas, Classified by Molecular Subtypes and Its Association with Prognostic Factors.
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Background: Breast carcinomas (BC) exhibit intra tumoral heterogeneity being stratified into several subgroups based in gene expression profiles or histochemical biomarkers. It was suggested that this heterogeneity is derived in part from the transformation of different subsets of cancer stem cells (CSC) in each intrinsic subgroup. The presence of CSC can be evidenced by phenotypic analysis of CD44 e CD24. This study aimed to identify the CD24 and CD44 immunophenotypes within invasive ductal breast carcinoma (IDC) subgroups defined by immunohistochemistry markers and determine its influence on prognosis as well as its association with the expression of Ki67, citokeratins (CK5 and CK18) and claudin-7.

Methods: Immunohistochemistry expression of CD44 and CD24 alone was investigated in 95 IDC cases arranged in a tissue microarray (TMA). The association with subgroups defined as luminal A and B;HER2 rich and triple negative, or with the other markers and prognosis was analyzed. Results: CD44+/CD24- and CD44+/CD24+ were respectively present in 8.4% and 16.8% of the tumors, a lack of both proteins was detected in 6.3%, while CD44+/CD24+ was determined in 45.3% of the tumors. Although there was no significant correlation between subgroups and different phenotypes, the CD44+/CD24- phenotype was more common in the basal subgroups but absent in HER2 tumors, whereas luminal tumors are enriched in CD44-/CD24+ and CD44+/CD24+ cells. The frequency of CD44+/CD24- or CD44-/CD24- have not been associated with clinical characteristics or biological markers. There was also no significant association of these phenotypes with the event free (DFS) and overall survival (OS). Single CD44+ was evident in 57.9% and was marginally associated to grading and not to any other tumor characteristics, while CD24+ was positive in 74.7% of the tumors, showing a significant association with ER, PR and Ki67 and a marginal association with CK18 and claudin-7. Expression of claudin-7 and Ki67 did not associate with the cancer subgroups, while a positive association between CK18 and the luminal subgroups was found (p=0.03). CD44+ was not significantly associated with OS and DFS whereas CD24+ frequency although not significantly associated with OS was associated with a decrease in DFS (p=0.07). CK5, CK18 and Ki67 expression had no influence in OS or DFS, claudin-7 positive was associated with reduced DFS (p=0.05). Conclusions: There was no significant correlation between CD44+/CD24+ tumor cells frequency and event-free or OS. However, a tendency toward a favorable prognosis, was noted. Contrariwise the presence of CD44+/CD24+ suggested a worse prognosis. Both single CD44 and claudin-7 positivity were associated with reduced time of recurrence, suggesting a contribution of these markers to aggressiveness. Supported by FAPESP and CNPq.

P5-11-17
Evaluating Tumor Heterogeneity in Immunohistochemistry Stained Breast Cancer Tissue.
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Context: Quantitative clinical measurement of heterogeneity in immunohistochemistry staining would be useful in both evaluating patient therapeutic response and identifying underlying issues in histopathology laboratory quality control.

Objective: To create a heterogeneity scoring approach (HetMap) that allows the visualization of an individual patient’s IHC heterogeneity in the context of a population.

Design: We combined HER2 semi-quantitative analysis with the use of ecology diversity statistics to evaluate cell-level heterogeneity (consistency of protein expression within neighboring cells in a tumor nest) and tumor-level heterogeneity (differences of protein expression across a tumor as represented by a tissue section). We evaluated the
P5-12-02
Vacuum Assisted Biopsies of Ductal Carcinoma In Situ and Concordance with Post-Operative Histology: Implications for the Low Risk DCIS Trial.

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Aim
The enormous increase in the diagnosis of ductal carcinoma in situ (DCIS) by the NHS Breast screening has not lead to an expected decrease in the incidence of invasive breast cancer. It is not clear if all grades of DCIS progress inexorably to invasive cancer if left untreated. There is recognition that DCIS is overtreated, if left alone may not cause harm during the woman’s lifetime. In the absence of new clinical trial data, surgery still remains the universal treatment. It is known that a higher proportion of patients with screen detected DCIS receive mastectomy than those with screen detected invasive cancer. Recently a randomized trial called the Low risk DCIS Trial has been proposed which intends to specifically compare the current treatment of low grade DCIS ie surgery with active monitoring using annual mammography. In order to effectively implement this, concordance between diagnostic biopsy and excision histology is vital and therefore vacuum assisted mammotome biopsy (VAB) and a central pathology review of diagnostic biopsy specimens prior to randomization will be mandatory. Therefore, in this study, we assessed the concordance between diagnostic biopsies performed by VAB technique and the post operative histology for DCIS in our institution.

Methods
Retrospective data of all diagnostic breast biopsies specifically using the VAB technique with the primary diagnosis of DCIS from year 2001 to 2010 in our institution was collected. Both screening and symptomatic patients were included. Concordance between diagnostic histology and post operative excision histology was assessed for high, intermediate and low grade DCIS. Demographic details and potential factors influencing concordance including number of cores taken and lesion size were also collected for analysis.

Results
A total of 161 cases were identified out of which 102 (63%) were of high grade, 35 (22%) of intermediate grade and 24 (15%) were of low grade histology. In the High grade group, the concordance with final histology was 70% (72/102). In this group, the diagnosis was upgraded to invasive carcinoma in 21% (21/102), 9% (9/102) were downgraded to intermediate or low grade. In the intermediate grade group, the concordance with final histology was 66% (23/35). In this group, the diagnosis was upgraded to invasive carcinoma in 11% (4/35) and to high grade in 17% (6/35). 6% (2/35) were downgraded to low grade. In the low grade group, the concordance with final histology was 71% (17/24). In this group, the diagnosis was upgraded to intermediate grade in 17% (4/24) and invasive carcinoma in 12% (3/24). All factors associated with lack of concordance were noted.

Conclusion
Concordance between VAB diagnostic biopsies of high, intermediate and low grade DCIS and post operative histology is good in this series and is to our knowledge the first to be reported using only large volume biopsies by VAB techniques. This audit has identified possible factors influencing the lack of concordance and these results with concordance data from other UK centres will be used by trial pathologists to refine protocols for the Low risk DCIS trial.
P5-12-03
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Background: Tamoxifen (TAM) has been commonly used as a selective estrogen receptor modulator for the treatment and prevention of breast cancer. Although this drug is effective in many patients, some develop resistant and eventually relapse. Recent studies suggest that the cancer recurrence could possibly be due to changes in drug metabolism. TAM is metabolized by the cytochrome P450 enzyme pathway into several metabolites including 4-hydroxy-tamoxifen (4-OH), N-desmethyl-tamoxifen (DMT) and N-desmethyl-4-hydroxy-tamoxifen (endoxifen). These metabolites have variable potencies in suppressing estrogen-dependent breast cancer. Differences in metabolism may contribute to the clinical inter-individual variability in TAM response. The aim of our study was to provide a comprehensive evaluation of TAM and its metabolites through quantitative measurement in breast cancer patients to help better understand the pharmacological effects of TAM therapy. In the past, most methods used to measure the levels of TAM and its metabolites were in plasma or fresh/frozen tissue samples. These samples are typically not available retrospectively, and their long-term storage is expensive and laborious.

Methods: We therefore explored the possibility to utilize formalin-fixed and paraffin-embedded (FFPE) tissues archived post breast surgery for quantification of TAM and its metabolites. Our laboratory has developed a rapid, sensitive and specific analytical method using liquid chromatography and tandem mass spectrometry (LC-MS/MS) for the measurement of TAM and its metabolites in FFPE tissues. The FFPE tissues were thin sectioned and deparaffinized by incubating twice with xylene for 10 min at room temperature. Sample clean-up was carried out subsequently using C2 solid-phase extraction, and detection was performed in the multiple-reaction monitoring mode with a triple quadrupole mass spectrometer. This method allows simultaneous quantification of TAM and three metabolites in FFPE tissues with a run time of 12 min.

Results: The assay had good inter- and intra-assay precisions (2-6 %CV), and was linear over the range of 0.01–5 ng/g for 4-OH and endoxifen, and 0.1–50 ng/g for TAM and DMT. The extraction recoveries were between 83-88%. The validated method was successfully applied to analyze the FFPE tissues obtained from two groups of breast cancer patients. Patients were categorized into those with tumor recurrence (R) and those without recurrence (NR) after at least 2 months of 20 mg/d TAM treatment. Levels of TAM, 4-OH, DMT and endoxifen in FFPE tissues were compared between the two groups. Our preliminary data show that the ratio of DMT/TAM was significantly higher in the R (6.7, n = 13) than the NR patients (14.2, n = 9) (p<0.05).

Discussion: The assay described here not only allows accurate quantification of TAM and metabolites in FFPE tissues, but also opens up an incredible opportunity and new challenges for researchers to excavate precious information from FFPE tissues, especially when these archival samples represent the only source of biomaterial available.

P5-12-04
Genetic Linkage between Acquired and Primary Lymphedema Evaluated through Whole Exome Sequencing and NIR Fluorescence Lymphatic Imaging.
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Acquired lymphedema is thought to arise from the damage of the lymphatic vasculature that transports excess fluid and macromolecules away from tissues for return to the blood vasculature. The onset of the cancer acquired disease can occur months to years after lymph node dissection and manifests itself as an accumulation of fluid and macromolecules in tissues that leads to edema and irreversible swelling. The rare disease of primary lymphedema is identical to cancer acquired lymphedema, with the exception that there is no trauma or cancer treatment that can be attributed as its cause. Primary lymphedema has been attributed to genetic causes since the late nineteenth century. Although there are five known genetic causes of hereditary or primary lymphedema, the majority of patients with lymphedema do not possess mutations in these genes. More recently, it has been proposed that a genetic link between cancer acquired and primary lymphedema exists. If a genetic susceptibility for cancer acquired lymphedema could be found, then we could predict which survivors will encounter the disease and could develop new therapies which are more effective than the current treatments that have remained unchanged for the past 80 years.

In an FDA approved investigational study, we used near-infrared (NIR) fluorescence imaging to phenotype the lymphatic architecture of subjects with both acquired and primary lymphedema, as well as their unaffected family members. We collected blood for DNA analyses. NIR fluorescence provided the phenotype of abnormal lymphatic function while whole exome sequencing provided the genotype. Bioinformatics analyses were then used to identify causative genes using cosegregation of familial genotypes using the phenotypes found through NIR fluorescence imaging.

The first family analyzed had members with primary and acquired lymphedema, in which mutations encoding for proteins that participate in the HGF/c-MET and PI3K pathways could potentially explain the inheritance of lymphedema in this family. The father and affected daughters were heterozygous for a de novo SNP HGF in the kringle binding domain that interacts with tyrosine kinase receptor c-MET. The father had a normal lymphatic phenotype. On the other hand, the mother and daughters were heterozygous for the de novo mutation of INPPL1 (SHIP-2), adjacent to the SH2 domain of the protein that is known to bind to the multifunctional docking site of c-MET and associates with proteins in the Rho pathway for cytoskeletal reorganization. The daughters possessed both HGF and INPPL1 mutations and were diagnosed with primary lymphedema while the mother, who possessed the INPPL1 mutation, was diagnosed at the time of NIR imaging with acquired lymphedema. Analyses of remaining families as well as breast cancer related lymphedema patients are underway to confirm whether INPPL1 may be a candidate susceptibility gene for acquired lymphedema. Supported in parts by R01 HL092923 and CA128919, The Texas Star Award, and the Cullen Foundation.
P5-12-05
(In-)Efficiencies in the Preoperative Imaging Evaluation of the Medicare Breast Cancer Patient.
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Introduction: Breast cancer evaluation requires a combination of physical examination and imaging for preoperative diagnosis and assessment of surgical treatment options. While imaging remains a critical component of that assessment, the burden of patient (pt) return visits for imaging is unknown.

Methods: Medicare claims linked to Surveillance Epidemiology and End Results data were reviewed for women developing breast cancer between 1992 and 2005. The preoperative interval was defined as the period from the first physician encounter for a breast-related diagnosis until therapeutic surgery. Pts without ≤6 mos of data prior to that interval, those having DCIS or Stage IV disease, and those having preoperative chemotherapy or radiotherapy were excluded. Imaging modality counts exclude image-guidance claims for biopsies.

Results: Among 353,265 Medicare pts developing breast cancer between 1992 and 2005, 67,751 women ≥65 y of age had invasive, nonmetastatic breast cancer, and simultaneous breast surgery (lumpectomy or mastectomy) with lymph node staging. Median age was 75 y and preoperative interval length was 27 d. In the 6 mos before the preoperative interval, mammograms (MMGs), breast ultrasounds (USs), and breast MRIs were performed exclusive of the preoperative interval in 34,192 (50.5%), 16,936 (25.0%), and 180 (0.3%) pts respectively, while during the preoperative interval, MMGs, USs, and MRIs were performed in an additional 30,414 (44.9%), 17,983 (26.5%), and 1,409 (2.1%) respective pts. Imaging was performed on ≥2 separate dates during the preoperative interval in 4.9% of pts in 1992, rising to 19.4% in 2005 (trend, p<0.0001). During that interval, there were ≥2 encounter dates for MMGs in 7.0% of pts, rising from 3.9% in 1992 to 8.8% in 2005 (trend, p=0.0001); for US, 3.6% overall, rising from 0.2% in 1992 to 6.6% in 2005 (trend, p<0.0001). Multiple MRI encounters were rare, occurring in 0.2% overall, and increasing to 0.6% in 2005 (trend, p=0.0001), while single MRI use increased from <0.1% in 1994 to 8.3% in 2005 (trend, p<0.0001). In the preoperative interval, use of more than one imaging modality on any given date increased from 4.3% in 1992, to 27.1% in 2005. Among those with imaging, there was low correlation between number of imaging dates and number of modalities on any given date (r = 0.13, p<0.0001). MMGs accounted for 71.9% of the days where one modality was performed alone, but MRI was performed alone 94.1% of the time. The total number of imaging dates have been increasing substantially since 1992, suggesting that the patient’s time burden for such evaluation is increasing. This trend is present despite the fact that multiple imaging modalities are being performed more frequently on the same date. Efforts to further consolidate preoperative breast imaging visits to lower that burden should be undertaken where possible in the Medicare population, for whom advanced age, in itself, may provide its own challenges.

P5-13-01
Survival Outcome with Bevacizumab: Activation of the Phosphatidylinositol-3 Kinase (PI3K) Pathway Due to PIK3CA Mutations or PTEN Loss Makes a Difference.
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Background: The PI3K pathway is known to regulate the transcription of Vascular Endothelial Growth Factor (VEGF) in endothelial and cancer cells. The inhibition of VEGF is the rationale behind the development therapy with anti-angiogenics. We investigated the activation of PI3K pathway, defined as PTEN loss and/or PIK3CA mutations, and the relationship with survival in metastatic breast cancer patients treated with the anti-VEGF monoclonal antibody bevacizumab.

Patients and methods: Records for patients with HER2 negative metastatic breast cancer (MBC), treated with bevacizumab from July 2005 to June 2010, and with known PI3K status were reviewed. Univariate and multivariate analysis were performed using SPSS 15.0.

Results: A total of 40 patients - 26 with and 14 without PI3K activation - were identified. Median age was 47 years (range 27-79). Median overall survival (OS) was 41.23 months (CI 95%: 27.29 – 55.18) and 66.23 months (CI 95%: 61.04 – 71.42) in patients with and without PI3K activation, respectively (p=0.04). Among patients with PI3K activation, OS was 34.13 months (CI 95%: 22.40 – 45.86) and 43.43 months (CI 95%: 26.9 – 59.97) in cases with PIK3CA mutations and PTEN loss, respectively. As compared to patients without PI3K activation, patients with PIK3CA mutations but not patients with PTEN loss showed a significant worse outcome (p=0.006 and p=0.3, respectively). In the multivariate analysis including grade, hormone receptor and Ki67 status, and total lines of treatment, the activation of PI3K activation proved to be an independent prognostic factor (p=0.03).

Conclusions: The activation of the PI3K pathway is significantly associated with decreased OS of MBC patients treated with bevacizumab. Prospective evaluation of PI3K activation on VEGF signaling with differential implication of PIK3CA and PTEN loss is warranted.

P5-13-02
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Background: Receptor tyrosine kinases and other membrane-associated tyrosine kinases are frequently overexpressed, activated, or mutated in cancer cells and cause aberrant signal transduction, which leads to cellular proliferation, angiogenesis, metastasis, and antiapoptosis. Blockage of these signals is considered an efficient strategy in cancer therapy, and to date, several tyrosine kinase inhibitors (TKIs) and antibody drugs targeting such kinases have been developed and are routinely used in clinics. However, the efficacy rates of the drugs are, unfortunately, limited. To provide optimum care for individual patients, an accurate prediction method for drug efficacy is now strongly demanded. Recent studies have shown that alteration of multiple kinases is involved in both primary and acquired drug resistance. Advantages of multi-targeted TKIs have also been
reported. These findings indicate the need for a comprehensive evaluation of multiple kinases for predicting drug response. To achieve this, we developed a novel method of comprehensively profiling kinase activity.

Materials and methods: We characterized the membranous tyrosine kinases of breast cancer cell lines using a newly established profiling assay. Briefly, crude membrane fractions were prepared and directly subjected to the assay with nonspecific substrate (Poly(Glu-7-Tyr)). The profile of the kinases in the crude membrane fraction was obtained by inhibiting the total activity with 13 selected kinds of adenosine triphosphate antagonists, independently. The residual activity (RA) of membranous kinases was defined as the percentage of activity with/without TKI.

Results: Nineteen breast cancer cell lines were classified as “sensitive” (n = 6) and “resistant” (n = 13) to dasatinib according to the definition in the publication (Cancer Res., 67 2226-38. 2007). The RA of tyrosine kinases targeted by different types of TKIs was determined by our assay, and we found that two RAs (src inhibitor 1 and PP1) showed statistically significant differences between the “sensitive” and “resistant” groups (p<0.017, and p<0.002, respectively, Student’s t-test). The RA of the two TKIs were also significant predictors for dasatinib sensitivity in a receiver operating characteristic curve analysis (area under the curve: 0.846 for src inhibitor 1 and 0.910 for PP1). Since the major target of dasatinib are src family kinases, protein expressions of the src family of the cell lines were quantified by a Western blotting and compared between the groups. We found that the protein expression is not a statistically significant predictor of dasatinib sensitivity.

Conclusion: We have shown that a comprehensive tyrosine kinase activity profiling assay of cancer cells can predict dasatinib sensitivity. We plan to validate this assay prospectively in patients with breast cancer who receive dasatinib.

P5-13-03
Correlation of Clinical and Molecular Findings with Pathologic Response to Preoperative Lapatinib and Trastuzumab, Separately or in Combination, Prior to Neoadjuvant Chemotherapy for HER2-Positive Breast Cancer

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Background: 15% of curative HER2-positive patients (pts) treated with standard trastuzumab (T)-based chemotherapy regimens recur. Our prior report (ASCO 2011 #506 using only data from paired samples N=49) identified major pathways of resistance: autophagy, stem cell, extracellular matrix, and phospho epitopes on FOXO, STAT3, EGFR.

Aim: To collect proteomic and microarray data from baseline biopsies and identify molecular correlates of pathologic complete response (pCR).

Methods: Randomized, open label, phase II. Eligibility: biopsy-proven HER2 positive stage II, III invasive breast cancer, healthy. After informed consent, pt randomized to T or Lapatinib (L) or both (T+L) for 2 weeks (wk) then continue same anti-HER2 agent with standard chemotherapy (FEC75 IV every 3 wk then Paclitaxel 80 mg/m2 wk x 12) then surgery. Research biopsy before and 2 wks after anti-HER2 agent analyzed by reverse phase protein microarray (RPMA) from laser capture microdissected cells, RNA expression microarray, and in-vitro stem cell studies. PCR in breast is no invasive tumor. ITT is Intent to Treat (all treated pts). Pathways identified by analytes (n=42): autophagy (Bcl-1, LC3B, AFG5), stem cell (CD44, Mushashi, E-Cadherin, Beta Catinin), extracellular matrix (Integrin, MMP). Additional informative analytes: phosphorylated HER3, IGFR1.

Results: 100 pts treated: T=33, L=34, T+L=33. % pCR-ITT: ER pos/ER neg by arm: T = 40%/67%; L = 21%/50%; T+L = 58%/50%. Baseline RPMA samples N= 65 (T = 22; L = 25; T+L = 18). A) Specific analytes contributory to outcome by arm by Random Forest analysis: F, Beta Catinin Ser 33/37/Thr 41, EGFR Tyr1045. L: MMP14, E-Cadherin, LC3B, Integrin alpha5B1, Stat 5 Tyr 694, IGFR Tyr 1131/1146, Erk Thr 202/Tyr204, NFKB Ser536, FOXO Thr24, HER3 Tyr1289. T+L: cerb2, EGFR, Met Tyr1235, CD44, E-Cadherin. B) Statistically significant endpoint ratios from network analysis that strongly correlate with pCR: F, HER3 Tyr1289/Beclin 1; PENTser380/ERK Thr202/Tyr204; GSK3B Ser21/Thr21/Stat5 Tyr694; 57065Thr389/LC3B; p7026 Thr389/MMP9; FOXO Thr24-32; NFKB Ser536; Beta Catinin Ser 33-37 Thr41/LC3B. L: EGFR Tyr1045/Beclin1; cerb2/Beclin1; HER3 Tyr1289/Beclin1; P43K/ FOXO Thr24, 32; IRS1 Ser612/Beclin1; Mushashi/IRS1 Ser612. T+L: only 4 pts no pCR. C) Microarray analysis confirms the critical role of extracellular matrix and autophagy.

Discussion: These findings support the concept that NO-pCR tumors can engage a diverse set of strategies to achieve cell survival in the face of therapy. The molecular correlates of response found in this small, underpowered study provide candidates for future patient stratification studies and generate insights for combination therapy.

P5-13-04
Changes in Phosphorylation Status at VEGFR2 and Basal Tumor Hypoxic Volume Assessed by Misonidazol (MISO) Positron Emission Tomography (PET/CT) as Potential Biomarkers for Predicting Response to Bevacizumab in Breast Cancer

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Background: To evaluate the expression of novel putative biomarkers as predictors of benefit from bevacizumab in stage II-III, previously untreated breast cancers (BC) patients (pts) in the context of a phase II, single-arm, multicenter and prospective clinical trial. To address this aim, we examined baseline and induced changes after a single bevacizumab administration as potential early predictors of response.

Methods: Pts received a single infusion of bevacizumab (15 mg/ kg) (C1) 3 weeks prior to the beginning of neoadjuvant chemotherapy consisting in 4 cycles of docetaxel (60 mg/mq), doxorubicin (50 mg/mq) and bevacizumab (15 mg/kg) every 21 days (C2-C5) following by surgery. Early assessment of tumor changes was performed by paired tumor-biopsies and MISO PET/CT before and after a single bevacizumab administration (C1). Biomarker expression was assessed by immunohistochemistry (IHC) (Ki67, CD31, CD31/Ki67, VEGFR2, pVEGFR2 [Y951]) on formalin-fixed, paraffin-embedded tissue before and after bevacizumab infusion (C1). MISO SUV and
tumor volume depicted by PET were calculated. Pathological response on surgical specimens was assessed according to Miller-Payne classification. Pts with tumor reduction >90% were considered as best responders (G4-G5) whether tumor reduction <90% were considered as non responders. Association between pathological response, IHC and MISO biomarkers was analyzed using Mann-Whitney test. ROC curve was performed to test sensitivity and specificity of the biomarker found significantly associated with response and its value as independent predictor was tested in the multivariate analysis using logistic regression.

Results: This analysis was performed on the training set including 73 patients (49 yr, range 29-70). Twenty (27%) patients obtained best response (G4-G5) whether 50 (68%) were considered as no responder (G1-G2-G3). Response was associated with negative estrogen receptors expression (p=0.02) and high Ki67 basal and after C1 expression (p=0.009 and p=0.01). Six (54%) of triple negative tumors were responders (p=0.05). Interestingly, change in pVEGFR2 [Y951] staining induced by bevacizumab administration and baseline MISO tumor volume was found significantly associated with response (p=0.03 and 0.057). Decrease in the phosphorylation status of VEGFR2 (Y951) >70% yielded a receiver operating characteristic (ROC) curve area of 0.681 (95% CI: 0.536 - 0.825) with 84% sensitivity and 95% specificity. The positive and negative predictive values for this marker were 60% and 64%, respectively. The change in phosphorylation status of VEGFR2p remains a significant predictor biomarker of response in multivariate analysis (OR=0.9, IC%95 0.96-0.99, p=0.04) after adjusting for clinical-pathological characteristics.

Conclusion: These findings underline the potential value of early change in phosphorylation status of VEGFR2 after bevacizumab infusion as predictive biomarker of response to anti-angiogenic therapy in breast cancer. Moreover, tumor hypoxic volume obtained by MISO might be associated with response. A validation set is warranted to confirm these findings.

P5-13-05
Non-Invasive In Vivo 1H Magnetic Resonance Spectroscopy (MRS) Monitoring of Breast Tumor Response to IGF1R Targeted Therapy.
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Drugs targeting IGF1R are in clinical trials for breast cancer therapy. To date no suitable biomarkers of response have been identified for this therapy. It is important to select patients with tumors sensitive to disruption of IGF1R signaling. In addition, predicting response in patients quickly following initial treatment would also assist in the development of these strategies. While expression of IGF1R must be a minimum requirement for enrolling patients in trials with IGF1R agents, the mere expression of IGF1R is not necessarily sufficient for IGF-driven biology. We have previously shown that monitoring levels of total choline-containing compounds (tCho) non-invasively in breast cancer patients with locally advanced breast cancer is a useful predictor of response to primary systemic therapy. The tCho measured in these studies included choline, phosphocholine (pCho) and glycerophosphocholine (GPC). In this study, we examined if changes in tCho could be used as a marker of response to anti-IGF1R therapy. We tested the effect of two antibodies against IGF1R on intratumoral tCho levels in vivo. Mice bearing MCF-7 xenografts were subjected to MR imaging (MRI) and spectroscopy (MRS) to measure baseline tCho levels. MRI was performed at 4.7 Tesla (200 mHz) with a transceive surface coil. Single-voxel 1H spectroscopy was performed using water suppression and TE-averaging. A fully relaxed, unsuppressed water spectrum from the same voxel was acquired and tCho signal was referenced against the tissue water signal and expressed as mmol/kg water. The next day, mice were treated with 500 µg of scFv-Fc or phosphate buffered saline, or 800 µg EM164 or isotype-matched control antibody. 24 hours post-treatment, tCho was measured. The percent change in tCho 24h after treatment (%tCho24) was determined using the formula 100[(tCho24-tCho0)/tCho0]. In 9 out 10 mice treated with scFv-Fc, %tCho24 ranged from -26 to -81% of baseline levels while in mice treated with PBS the change was from 0% to +46%. EM164 treatment also caused decrease in tCho levels in 3 out of 3 mice while the control antibody did not. tCho was also monitored for a period of 2 weeks during the course of treatment with EM164 or control antibody; EM164 caused a decrease in tCho with %tCho of -35% while the control antibody treated mice showed %tCho of +43% over the two-week treatment. To further delineate the precise choline species regulated by IGF signaling, MCF-7 cells were treated in vitro with IGF-I for 24h and cell extracts subjected to MR at 14.1 Tesla using a NMR scanner. IGF-I enhanced levels of pCho compared to choline and GPC. We next investigated if there is a mechanistic link between IGF signaling and choline metabolism. MCF-7 cells were treated with IGF-I for 10 minutes or 24h and levels of choline kinase (ChoK), the rate-limiting enzyme that converts choline to pCho and then phosphorylcholine were examined. Surprisingly, IGF-I signaling enhanced ChoK levels. Our data show that decrease in tCho could be a surrogate marker of early response to anti-IGF1R therapy. Thus, MRS to measure tCho levels could be included in the design of clinical trials with such reagents to quickly identify patients whose tumors are sensitive to inhibition of IGF1R.

P5-13-06
Lipidomic Profile Predicts Pathologic Complete Response to Neoadjuvant Weekly Paclitaxel Treatment.
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BACKGROUND: Neoadjuvant chemotherapy is one of the main strategies for patients with advanced breast cancer. The taxane paclitaxel have an important role in inhibiting microtubule polymerization and this mechanism prevents cells from entering into the mitotic phase. The therapeutic efficacy and toxicity profile of weekly paclitaxel administration have been showed since 90s. Lipidomics may be defined as the large-scale study of pathways and networks of cellular lipids that is involved in biological systems. In the present study our aim was to evaluate the possibility to identify a lipidomic signature of good and bad responders to neoadjuvant chemotherapy.

PURPOSE: To analyze and identify the lipid fingerprint in tumor biopsy of advanced breast cancer patients who get a complete pathologic response (CPR) with weekly paclitaxel treatment and compare this with the group who get no response.

MATERIAL AND METHODS: Seventy eight patients with clinical stage IIIA breast cancer were selected to receive the neoadjuvant into the mitotic phase. The therapeutic efficacy and toxicity profile of weekly paclitaxel have an important role in inhibiting microtubule polymerization and this mechanism prevents cells from entering into the mitotic phase. The therapeutic efficacy and toxicity profile of weekly paclitaxel administration have been showed since 90s. Lipidomics may be defined as the large-scale study of pathways and networks of cellular lipids that is involved in biological systems. In the present study our aim was to evaluate the possibility to identify a lipidomic signature of good and bad responders to neoadjuvant chemotherapy.

PURPOSE: To analyze and identify the lipid fingerprint in tumor biopsy of advanced breast cancer patients who get a complete pathologic response (CPR) with weekly paclitaxel treatment and compare this with the group who get no response.

MATERIAL AND METHODS: Seventy eight patients with clinical stage IIIA breast cancer were selected to receive the neoadjuvant treatment with weekly paclitaxel (for a total of 12 doses – 80mg/m2). Fifty eight patients completed the treatment purpose. Seven presented pathologic response (CPR) with weekly paclitaxel treatment and 5 showed progressions (Group B). The lipids were extracted from biopsies and submitted to matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS). MALDI-MS spectra were acquired in the positive ion and reflection modes using Synapt Q-ToF mass spectrometers (Waters, Manchester, UK). The spectra were acquired in the m/z 700-1200 range. From each spectrum...
the most intense ions were considered as the starting point of searching m/z values which were clearly distinct from noise level in the spectra were included in the principal component analysis (PCA).

**RESULTS:** The PCA analysis of the mass spectra shows that groups can be resolved via their MALDI-MS lipid profiles, which suggest the patients who presented CPR can present distinct lipid fingerprint in the tumor biopsy compared to patients who showed progressions.

**CONCLUSION:** After genomic and proteomic innovations, it turns necessary to explore metabolic processes at the system level. Lipidomics is a developing strategy for functional genomics, since there is growing evidence that lipids are structural biomolecules, which have important function as signal transduction processes, second messenger molecules or their precursors. Is possible that lipidomic profile of tumor specimen could help us to select the patients with advanced breast cancer that might present complete pathologic response by weekly paclitaxel treatment. This study will be continued to confirm this results and carried the identification of the lipids based on earlier studies and MS/MS data using lipidmaps and chemspider databases.

**P5-13-07**

**MET and Hepatocyte Growth Factor (HGF) Increased Gene Copy Number Is Associated to Trastuzumab Failure in HER2 Positive Metastatic Breast Cancer (MBC).**

Minuti G, Duchnowska R, Jassem J, Roncalli M, O'Brien T, Fabi A, Landi L, Di Marsico R, Biernat W, Czartoryska-Arlukowicz B, Jankowski T, Zaciak D, Zok J, Szostakiewicz B, Foszczyńska-Kloda M, Tempinska-Szalach A, Rossi E, Varella-Garcia M, Cappuzzo F. Istituto Toscana Tumori, Civil Hospital of Livorno, Livorno, Italy; Military Institute of Medicine, Warsaw, Poland; Medical University of Gdańsk, Gdańsk, Poland; Milan University, Istituto Clinico Humanitas, Milan, Italy; Laboratory of Molecular Pathology, University of Colorado Cancer Center, Denver, CO; National Cancer Institute Regina Elena, Rome, Italy; Białystok Oncology Center, Białystok, Białystok, Poland; Lublin Oncology Center, Lublin, Poland; Beskid Oncology Center, Bielsko-Biała, Poland, Bielsko-Biała, Poland; Warmia and Mazuria Oncology Center, Olsztyn, Poland; West Pomeranian Oncology Center, Szczecin, Poland; District Hospital Ełbląg, Ełbląg, Poland; CINECA Interuniversity Consortium, Bologna, Italy

Background: The ErbB2-targeting monoclonal antibody trastuzumab has remarkable efficacy in metastatic breast cancer (MBC) patients (pts) with HER2 overexpression or amplification (HER2+), either alone or in combination with chemotherapy. However, the response rate to trastuzumab is modest and not all pts derive benefit from this treatment. Predictive mechanisms of sensitivity and/or resistance are largely unknown. Recently, preclinical and limited clinical data showed that aberrant MET expression in MBC is a predictor of poor prognosis and is involved in trastuzumab resistance. Aim of the present study was to investigate whether increased gene copy number of MET or its ligand, the hepatocyte growth factor (HGF), affect trastuzumab sensitivity.

**Patients and Methods:** This retrospective study included 130 HER2+ MBC pts treated with trastuzumab as monotherapy (N=21) or in combination with chemotherapy (N=109). Main inclusion criteria were presence of at least one measurable lesion and availability of paraffin-embedded tumor tissue from primary cancer. **MET and HGF** gene copy number (GCN) were assessed by fluorescence in situ hybridization (FISH). Receiver operating characteristic (ROC) analysis was used for identifying the best **MET** and **HGF** mean GCN cut-off.

Results: In the whole population response rate (RR), including complete (CR) and partial response (PR) was 49.2%, disease control rate, including CR+PR+ stable disease (SD) was 76.2%, median time to progression (TTP) 9.4 months, and median survival (OS) 28.3 months. MET FISH analysis was successfully performed in all 130 cases. Median MET mean GCN was 2.96 (range 1.66-8.40), with no gene amplification. ROC curve identified a mean of 3.72 MET GCN as the optimal cut-off value for discriminating between sensitive (CR+PR+SD) and refractory pts (pts with progressive disease [PD]) at the first disease assessment. **MET** FISH+ (N=36, mean ±3.72) had a significantly higher PD rate (44.4% versus 16.0%; p=0.001) and a significantly shorter TTP (5.7 versus 9.9 months; HR 1.74 95% C.I. 1.16-2.62; p=0.006) than **MET** FISH- pts (N=94, mean ±3.72). HGF GCN was successfully evaluated in 84 pts (64%). Median HGF mean GCN was 2.80 (range 1.14-6.90). ROC analysis identified a cut-off of 3.01 mean HGF GCN as the best discriminating between sensitive (CR+PR+SD) and refractory pts. **HGF** FISH+ (N=33, mean ±3.01) had a significantly higher PD rate (30.3% versus 7.8%; p=0.007) and a non-significant shorter TTP (9.9 versus 10.5 months, HR 1.10 95% C.I. 0.70-1.74; p=0.66).

Conclusions: High GCNs of **MET** or **HGF** associate with an increased risk of trastuzumab failure in HER2+ MBC. These data support a further development of combining anti-HER2 with anti-MET strategies in MBC.

**P5-13-08**

**Breast Cancer Index Predicts Likelihood of Breast Conservation Surgery after Neoadjuvant Chemotherapy.**


Introduction: Neoadjuvant chemotherapy increases the likelihood that breast conservation therapy for breast cancer patients will be successful. Breast Cancer Index (BCI), a gene expression assay combining HoxB13/IL17BR ratio and Molecular Grade Index (MGI), is prognostic for the risk of distant recurrence and overall survival in tamoxifen-treated and untreated breast cancer patients. It was previously reported that high risk patients, as determined by BCI, had a 10-fold greater probability of pathologic complete response (pCR) with neoadjuvant chemotherapy than low risk patients. The aim of the current study was to examine the relationship between BCI score and the use of breast conservation surgery (BCS) following treatment with neoadjuvant chemotherapy.

Material and Methods: A total of 145 women (tumor size T1, T2 and T3) were treated with neoadjuvant chemotherapy for stage I-III breast cancer. RNA was extracted from FFPE tumor samples and a real-time RT-PCR assay was completed to generate a BCI score and risk group categorization as previously described (Jerevall et al. Br J Cancer 2011). The relationship between BCS, BCI and clinicopathological factors was examined using univariate and multivariate logistic regression.

Results: Of the 145 patients (67% ER+, 54% PR+, 57% >50 y old), 48 (33.1%) underwent BCS. BCI categorized 62 (43%) of patients as low, 50 (34%) as intermediate and 33 (23%) as high risk. The rate of BCS for the three BCI risk categories was 15% (low risk), 48% (intermediate risk) and 45% (high risk). In the low risk group, the rate of BCS was 15% corresponding to a NPV of 85%. This is consistent with previous data from the same cohort, where the NPV of BCI for pCR was 98.4% with only one patient in the low risk group achieving pCR. In univariate analysis, pathological tumor size (pT), ER, PR, grade and BCI were predictors of BCS. A higher BCI score
was associated with higher likelihood of BCS (odds ratio of 3.90; CI: 1.45-10.49; p=0.0069). In multivariate analysis, pT and BCI remained significantly associated with BCS, while ER status was not (p=0.23). Results were similar in the subset of patients with T1 and T2 tumors (N=97). In this subset, BCI categorized 42% of patients as low, 37% as intermediate and 21% as high risk and the rate of BCS was 22%, 64% and 60%, respectively. In multivariate analysis of this subset, only BCI was significantly associated with BCS. In all patients, the concordance index based on a model with pT alone was 0.695. When BCI was incorporated into the model with pT, the concordance index increased to 0.801 (p = 0.0002).

Conclusion: In this study, we have shown that patients with higher BCI scores were associated with a higher likelihood of receiving BCS after neoadjuvant chemotherapy. Addition of BCI to tumor size increased accuracy in predicting likelihood of BCS. BCI along with standard pathological factors may improve estimation of individual probability of BCS after neoadjuvant chemotherapy. This study gives rise to the hypothesis that patients with low BCI should not be eligible for neoadjuvant chemotherapy since the likelihood of breast conservation is low. Further large confirmatory studies are necessary.

### P5-13-09

**Correlation of OncoType DX Recurrence Scores with Pathologic Response Following Neoadjuvant Ixabepilone and Cyclophosphamide in Patients with HER2-Negative Breast Cancer: A Sarah Cannon Research Institute Phase II Trial.**

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Background: Ixabepilone (Ixa) is active in anthracycline and taxane-refractory metastatic breast cancer as well as in the neoadjuvant setting where Ixa yielded a pathologic complete response (pCR) rate of 11%. In this study, we evaluated Ixa in combination with cyclophosphamide (C) as neoadjuvant treatment for ER+/HER2-negative breast cancer. The primary endpoint was pathologic complete response (pCR) rate, defined as no residual invasive cancer in breast or lymph nodes. The Oncotype DX Breast Cancer Assay, that uses reverse transcriptase-polymerase chain reaction (RT-PCR) to assess a panel of 21 tumor genes to calculate a score predictive of the likelihood of the magnitude of adjuvant chemotherapy benefit, was applied to pretreatment and residual disease breast cancer tissues obtained at the time of definitive surgery. The likelihood of whether neoadjuvant clinical or pathological responses may be predicted by Oncotype DX recurrence scores (RS) was assessed. An interim analysis of the first 81 patients (pts) was reported at ASCO 2011. The final analysis will be presented on all 168 pts.

Methods: Eligible women had locally advanced breast cancer that was HER2-negative (IHC 0-1+ or FISH negative). T >2 cm or lymph node positive. Pts with inflammatory breast cancer or T1N0 tumors were excluded. Pts received Ixa 40mg/m² with C 600mg/m² day 1 q21 days x6. Following 6 cycles, had definitive surgery. Postoperative locoregional radiation therapy and/or hormonal treatments were at the discretion of the treating MD per institutional guidelines. Breast core biopsy tumor samples were obtained pretreatment and at the time of surgery in those pts demonstrating residual disease at surgery. Tumor specimens were analyzed using the Oncotype DX RT-PCR assay. An interim pretreatment RS assessment in the first 38 pts and paired samples in 21 pts was conducted and correlated with clinical and pathologic responses.

Results: 168 women enrolled. Baseline characteristics and toxicity for the first 118 pts are reported (median age 52 years; 90% invasive ductal carcinoma; T2/T3 52%/31%; 42% triple negative). 81 pts have undergone surgery. 25 pts discontinued treatment early (toxicity – 12; disease progression – 8; pt/MD request – 3; pt non-compliance - 2). Grade 3/4 toxicity included: neutropenia (65%), leukopenia (47%), neuropathy (10%), and febrile neutropenia (7%). Preliminary toxicity results with this neoadjuvant treatment have been previously reported (Peacock et al, ASCO 2011 Abstract #1066). OncoType DX pretreatment evaluations for the initial 38 pts demonstrated a significant logistic regression of pretreatment recurrence score with pCR (p = 0.025).

Conclusions: Neoadjuvant therapy with Ixa and cyclophosphamide yielded a preliminary pCR rate of 19% (in 81 pts), similar to results with other 2-drug combination chemotherapy regimens. Preliminary OncoType DX assessments at baseline indicated that baseline recurrence scores may predict pCR rates. These exploratory Oncotype DX recurrence scores at baseline and paired with scores obtained at the surgery and correlations with pCR will be presented for the entire cohort.

### P5-13-10

**Discordance of Estrogen Receptor and HER-2/neu Status Can Be Seen between First and Subsequently Biopsied Metastatic Lesions in Breast Cancer.**

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Background: We have previously reported discordance between primary and first metastasis for both estrogen receptor (ER) and HER-2/neu (HER 2) status (Breast Cancer Res Treat 2005;90:65-70 and Breast Cancer Res Treat 2009;113:301-6). Because biomarker status is the primary determinant for targeted therapy, changes in biomarkers can impact recommendations for systemic treatment.

Materials and Methods: In order to evaluate the possibility of further discordance with the development of additional metastatic sites, we examined the ER and HER 2 status of primary and first and subsequently biopsied breast cancer metastasis. In most cases, the subsequent site of metastasis was the second detected metastasis. Pathology reports were reviewed from 65 patients with biopsy proven first and additional metastasis; however, immunostain results were not available for all three timepoints for all patients. Sites of biopsied metastasis included lung, liver, bone, chest wall, and central nervous system.

Results: The time between the initial diagnosis and first metastasis was 102 (3-676) months [Median (Range)], and the time between the first and subsequent metastasis was 60 (6-273) months. For ER, there was discordance between the primary lesion and either the first or subsequent metastasis in 18 of 65 cases (27.7%). Discordance of the ER occurred between the first and subsequent metastasis in 12 (18%) of cases. Three patterns of ER discordance occurred in these 12 cases: 4 cases of both primary and first metastasis negative with subsequent metastasis positive; 3 cases of primary positive, first metastasis negative, and subsequent metastasis positive, and 5 cases of primary and first metastasis positive and subsequent metastasis negative. For HER 2, discordance was detected between the primary and either first or subsequent metastasis in 11 of 36 cases (30.6%). Further discordance between the first and subsequent metastatic lesions occurred in 8 of 51 (16%) of cases.
Conclusions: A significant level of discordance for either ER or HER 2 was detected between first and subsequent metastatic breast cancer lesions. For breast cancer patients with new metastasis, consideration of reevaluating ER and HER-2/neu status may be worthwhile.

P5-13-11  
PTEN and Tau-Protein Expression: Predictive Value of Poor Response to Trastuzumab Plus Paclitaxel in Patients with HER2-Positive Breast Cancer.  
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Backgrounds: Trastuzumab-based chemotherapy has been an active treatment in patients (pts) with HER2-positive breast cancer; however, primary and secondary resistance has occurred in pts treated with trastuzumab (H) alone or in combination with chemotherapy. Material and Methods: Biomarkers were searched using tissue microarrays (TMA) in the HER2-positive breast cancer pts treated with H and paclitaxel (P) combination chemotherapy between October 2004 and August 2010. Tumor blocks of 101 pts were analyzed for VEGF, IGF-1R, p-Akt, beta-III tubulin, CD44, Tau-protein, p27 and PTEN by immunohistochemical (IHC) analysis. Eight biomarkers were assessed to investigate the correlation with the clinical outcomes, including response rate (RR), progression free survival (PFS), and overall survival (OS).  
Results: With a median follow-up duration of 21.7 months (range, 9.1-55.2 months), 101 pts received H+P chemotherapy in neoadjuvant setting (n=36, 35.6%) and recurrent or metastatic setting (n=65, 64.4%). Median age was 48 (range, 19-83 years), and the majority of pts (n=95, 94.1%) had good performance status. Premenopausal pts and hormone receptor-negative pts were 48 (47.5%) and 52 (51.5%), respectively. The median cycle of H+P chemotherapy was six (range, H 1-43; P 1-21). Overall RR was 68.3% (n=69) including complete response with 7 pts, and PFS and OS were significantly longer in pts responsive to H+P chemotherapy compared with non-responsive patients (PFS, p=0.001; OS, p=0.015). Although VEGF, IGF-1R, p-Akt, beta-III tubulin, CD44, Tau-protein, p27 and PTEN status by IHC were not significantly associated with response to H+P chemotherapy, Tau-protein showed a trend of association without statistical significance (RR, 46.2% vs. 71.6%, p=0.066). Among 13 pts with high Tau protein expression, 9 pts with both high Tau-protein and low PTEN level showed statistically significant lower RR compared with other 92 pts (22.2% vs. 72.8%; p=0.002). None of the biomarkers was related to PFS and OS in pts with recurrent or metastatic disease, and to pathologic complete response in pts after H+P chemotherapy as neoadjuvant therapy. Conclusion: Our data showed that both low PTEN level and high Tau-protein expression were significantly associated with poor response to H+P chemotherapy in patients with HER2-positive breast cancer.

P5-13-12  
Can the Ki67 Proliferation Index Predict the Oncotype DX Recurrence Score in Lymph Node Negative, ER Positive Breast Cancer?  
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Background Oncotype DX is a 21-gene assay for node-negative, ER positive breast cancer. Results are expressed as a Recurrence Score (RS). This predicts a low, intermediate or high risk of distant relapse. Proliferation-related genes such as Ki67 impact heavily upon the calculation of the RS. The assay encompasses several histopathologic factors (ER, HER2) and is limited by cost, proprietary nature and turn-around time. We investigated whether Ki67 proliferation index (PI) and routine clinicopathologic parameters could be used to predict RS.  
Material and Methods We obtained clinicopathologic details for 69 patients (diagnosed 2007-2010) who had RS available. Quantitative analysis of Ki67 immunohistochemistry was performed on primary tumour. Patients were classified as having a low (≤15%), intermediate (16-30%) or high (>30%) Ki67 PI. Risk of recurrence was calculated using Adjuvant! Online. Patients with categorized as having a poor, moderate, good or excellent prognosis as per the Nottingham prognostic index (NPI) and a low, intermediate or high risk of relapse, according to the St. Gallen criteria.  
Results Mean age at diagnosis was 51.4 years (range: 34-74 years). Mean tumour size was 1.9 cm (range: 0.8-4.1cm). Mean Ki67 PI was 13.4 % (range 0.2 to 52.6%). RS was low (0-17) in 34 cases, intermediate (18-30) in 26 cases and high (>30) in 9 cases. The RS was significantly correlated with Ki67 expression (Spearman’s rank correlation, r = 0.472, p=0.001). RS was significantly higher in patients with a high Ki67 PI, when compared to patients with low and intermediate Ki67 PI (Mann Whitney U, p=0.05). RS was significantly higher in patients with grade 3 tumours, when compared to patients with grade 1 and 2 tumours (Mann Whitney U, p<0.05). Risk of recurrence as predicted by Adjuvant! Online, was significantly higher in patients with a high RS, when compared to patients with low and intermediate RS (Mann Whitney U, p<0.05). RS was significantly higher in patients with moderate prognosis, as determined by the NPI, when compared to patients classified as having a good/excellent prognosis (Mann Whitney U, p<0.05). No patients were classified by the NPI as having a poor prognosis. RS was significantly higher in patients deemed to be at high risk of relapse by the St. Gallen criteria, when compared to patients classified as low and intermediate risk (Mann Whitney U, p<0.05). The majority (66.7%) of cases in the high RS group had LVI present.  
Conclusions This pilot study indicates a trend whereby Ki67 and grade will predict whether the Oncotype DX RS is low/intermediate or high. Adjuvant! Online, St. Gallen and NPI are also able to distinguish patients with a high RS from those with low/intermediate RS. In order to verify these findings, we are developing a statistical model using these 69 patients as a training set. As there is insufficient follow-up to correlate either RS or Ki67 with relapse, a potential test set comprised of an additional 50 LN negative, ER positive patients, with an average of 13 years follow-up, has been identified. Ki67 analysis of these cases is underway to address this issue.  
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P5-13-13  
The Role of Topoisomerase IIα in Predicting Sensitivity to Anthracyclines in Breast Cancer Patients: A Meta-Analysis of Published Literatures.  
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Topoisomerase IIα is not only a proliferation marker of tumor cells but also a target for anthracycline-based chemotherapy. Both in vitro and in vivo studies have shown that there was a relationship between topo IIα and chemosensitivity to anthracyclines, but the predictive role of topo IIα is still controversial in breast cancer patients. A
meta-analysis based on published studies was performed with the aim of obtaining an accurate evaluation of the association between topo IIα and sensitivity to anthracycline-based chemotherapy. A total of 13 eligible studies including 2,633 cases and 2,118 controls were identified. Topo IIα was associated with sensitivity to anthracyclines in locally advanced breast cancer patients who received neoadjuvant chemotherapy (RR = 1.93, 95%CI: 1.27-2.94, P=0.002; RR =1.98, 95%CI: 1.37-2.86, P<0.001). In early breast cancer patients who received anthracycline-based adjuvant chemotherapy compared with non-taxane-based polychemotherapy, amplification (HR = 0.64, 95%CI: 0.49-0.83, P=0.001; HR = 0.59, 95%CI: 0.35-1.01, P=0.056) or deletion (HR = 0.82, 95%CI: 0.67-1.00, P=0.051; HR =0.58, 95%CI: 0.35-0.97, P=0.036) of topo IIα was significantly associated with better RFS and OS. The subgroup analysis in the early breast cancer patients indicated that taxane could be an interference for evaluation of the predictive role of topo IIα. In summary, the present meta-analysis suggests that topo IIα is a predictive factor for breast cancer patients who received anthracycline-based chemotherapy. Larger and well-designed prospective studies are required to further evaluate the predictive role of topo IIα in clinical practice.

P5-13-14
Drug-Metabolizing Enzyme Polymorphisms and Clinical Outcome of Anthracycline-Based Chemotherapy in Chinese Han Breast Cancer Patients.
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Background: Anthracycline is one of the most effective drugs in the treatment of breast cancer and many of our most effective treatment regimens include anthracycline. Its efficacy can be influenced by cellular detoxification mechanisms involving drug metabolism and transport pathways. This study aimed to assess whether the functional polymorphisms in drug-metabolizing enzymes (MnSOD, CAT and GSTs) and transporter MDR1 may predict anthracycline treatment-related outcomes in Chinese Han breast cancer patients.

Material and methods: Genotyping was performed by allele-specific oligonucleotide ligation reaction (MnSOD T47C, CAT C-262T, GSTP1 A131G), multiplex PCR (GSTM1 null, GSTT1 null), and PCR-RFLP (MDR1 C3435T, G2677T/A and C12367). Based on 153 evaluable patients received anthracycline-based neoadjuvant chemotherapy for breast cancer, the associations of these genotypes or their haplotypes with clinical response and recurrence-free survival (RFS) were analyzed.

Results: Of the 153 cases, the patients with GSTP1 313AA genotype had inferior response rates relative to those with AG or GG genotype (58.4% vs 77.8% or 100.0%; χ2=4.922, P=0.027). Moreover, the response rate of the combination of GSTP1 AA with both GSTT1 present and GSTM1 present was 44%, which was also lower comparing with the other groups (70.3%; χ2=6.454, P=0.011). A similar result was noticed for MDR1 3435 TT genotype, which had a significantly worse chemotherapy response compared with wild-type Callele carrier (33.3% vs 71.2%; χ2=11.586, P=0.001). Further, the response rate of the patients with 3435T-2677T, 3435T-12367T or 3435T-2677T-12367T haplotypes was lower than that of the patients with the other corresponding haplotypes (P=0.018, 0.011 and 0.019, respectively). Too. Of note, the patients with both the adverse genotypes of GSTP1 314AA and MDR 3435TT showed the worst treatment efficacy in all (14.3%; χ2=26.33, P=0.000). Mean follow-up time of the 149 patients (4 patients lost) was 51 months. The recurrence rate in the patients with GSTP1 313AA or and MDR 3435TT in the first three years was 39.0% (41/105), higher significantly than those with no adverse genotype [15.9% (7/44); OR=0.725, 95%CI: 0.594-0.885, P=0.006]. Kaplan-Meier survival analysis showed that the patients with no adverse genotype were associated with reduced hazard of relapse (long-rank test, P<0.01), compared to those with 1 or 2 adverse genotypes.

Conclusion: Polymorphisms in GSTs and MDR1 genes may help to predict clinical response and RFS of anthracycline-based chemotherapy in breast cancer, but further validation is required. These results provide support for a polygenic pathway approach for assessing the predictive potential of polymorphisms in treatment outcomes.

P5-13-15
Proteomic Identification of Predictive Biomarkers of Resistance to Neoadjuvant Chemotherapy in Luminal Breast Cancer: A Possible Role for 14-3-3 and BID?
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Background: Chemotherapy resistance is a major obstacle in effective neoadjuvant treatment for oestrogen receptor (ER)-positive breast cancer. The ability to predict tumour response would allow chemotherapy administration to be directed towards only those patients who would benefit, thus maximising treatment efficiency. We aimed to identify protein biomarkers associated with chemotherapy resistance, using proteomic analysis of fresh ER-positive breast cancer samples, and then to perform pilot clinical validation experiments.

Materials and Methods: Chemotherapy resistant and chemotherapy sensitive tumour samples were collected from breast cancer patients who received standard anthracycline-based neoadjuvant therapy consisting of epirubicin with cyclophosphamide followed by docetaxel. Comparative proteomics experiments were performed using invasive ductal carcinomas which demonstrated ER-positivity (luminal subtype). Protein expression was compared between chemotherapy resistant and chemotherapy sensitive tumour samples using 2-dimensional gel electrophoresis (2-DE) with MALDI-TOF/TOF mass spectrometry (MS). In addition the Panorama XPRESS Profiler725 antibody microarray, containing 725 antibodies from a wide variety of cell signalling and apoptosis pathways, was employed in the discovery phase. Differentially expressed proteins (DEPs) were submitted to Ingenuity Pathway Analysis (IPA) to identify any canonical pathway links. A pilot series of archival breast cancer samples, from patients treated with neoadjuvant anthracycline-based chemotherapy, was used for preliminary clinical validation of putative predictive biomarkers.

Results: Five datasets were generated by antibody microarray analysis, revealing 41 targets. Of these, 7 DEPs were identified in at least 2 datasets and these included 14-3-3, BID and Bcl-xL. The top canonical pathway matched in IPA was “ERK5 signaling”, which involved 6 DEPs, including 14-3-3. The “PI3/ekt” pathway also involved 6 DEPs, including 14-3-3 and Bcl-xL. Three datasets were generated using 2-DE with MALDI-TOF/TOF MS, containing over 300 DEPs. These included several isoforms of 14-3-3. Differential expression of 14-3-3, BID and Bcl-xL was confirmed by immunoblotting in samples used for the discovery phase. A pilot clinical validation using immunohistochemical analysis of archival breast cancers revealed 14-3-3 tau and tBID to be significantly associated with chemotherapy resistance.

Discussion: We have successfully utilised clinical tumour samples for the discovery of putative biomarkers of chemotherapy resistance using...
two complementary proteomic platforms. We propose a potential role for 14-3-3 tau and BID as predictive biomarkers of chemotherapy resistance in ER-positive tumours and further validation in a larger sample series is now required.

**P5-13-16**

Reduction in PET Uptake-Value Is an Early Predictor for Response to Neoadjuvant Therapy Including Anthracycline and Taxane in Stage II-IIIA Breast.

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**Background and Aim:** Reduction in FDG-PET uptake as response predictor after induction chemotherapy for breast cancer has been described. Our aim was to determine whether a SUV reduction ≥45% in the breast lesion could accurately predict pathological Complete Response (pCR).

**Material and Method:** Stage II-IIIA breast cancer patients (p) with primary tumor ≥3cm or positive axilla were included provided they were amenable to neoadjuvant chemotherapy with Docetaxel-Doxorubicin-Cyclophosphamide (TAC). A FDG-PET scan was performed on day -1 of the first course and was repeated on day+8 of the same course. SUV-uptake differences (day 8 minus day -1) were correlated to the eventual pathological response according to Miller-Payne criteria.

**Results:** 42p were included. Median age was 45 years (30-66). 11p (26.2%) were triple-negative tumors. 2p (4.8%) were Her2+. By stage, 13p (30.9%) were IIA, 17p (40.4%) IIB and 12p (28.2%) IIIA. 23p (54.7%) were N+ (FNAB positive). Median breast-SUV on first PET scan was 5.51 (1.8-18.50). 40p (95.2 %) completed the scheduled 6 courses. 41p (97.6%) were submitted to surgery. 11p (26.2%) achieved a pCR. 40p (95.2%) were evaluable for the primary objective. 1p (0.02%) died of disease progression before surgery and 1p (0.0%) with pCR could not be assessed by PET. 7 out of 14 p (50%) with uptake reduction ≥45% with respect to the basal value, and 3/ 26p (11.5%) whose reduction was <45% achieved a pCR (p=0.007). SUV decrease >45% accurately predicted 7/ 10 pCR (70%) that could be assessed.

**Conclusions:** Our study confirmed the value of PET-SUV reduction on day 8th as an early predictor of pCR with neoadjuvant TAC chemotherapy.

**P5-13-17**

Multigene Signature Assays in Patients with Early-Stage Breast Cancer (ESBC) Receiving Neoadjuvant Chemotherapy: An NCI-Funded Systematic Review and Evidence Summary of Predictive Performance.

Lyman GH, Cukalova E, Poniewierski MS, Huang M, Barry W, Ginsburg G, Abernethy A, Marcom PK, Ready N, Kuderer NM. Duke University School of Medicine, Durham, NC; Duke University, Durham, NC

Background: A comprehensive literature search and evidence synthesis of multigene signatures predictive of response to systemic chemotherapy in patients with breast cancer was initiated as a part of an NCI-funded program on Comparative Effectiveness Research. Methods: Validation studies were sought of multigene signatures for prediction of chemotherapy response (favorable vs unfavorable) in ESBC patient cohorts different from those used for signature development. Pooled estimates [≥95% CI] of assay performance for predicting clinical outcome included sensitivity, specificity, likelihood ratio, predictive value (PV) and predictive odds ratio (POR) utilizing mixed effects models based on the method of Mantel-Haenszel. Exploratory metaregression analyses on log (POR) were also performed. Studies were classified by validation type including cell lines to patients, independent internal sample, random split sample, or external validation. Evidence for publication bias was assessed by Egger’s regression intercept and Begg and Mazumdar’s rank correlation. Results: Dual-blind review of abstracts identified 33 studies of neoadjuvant chemotherapy response of which 29 stratified treatment response by signature classifier category. Classifier development was based on tumor response prediction in 20 studies, prognosis in 5, and molecular classification in 4. The Table shows assay performance measures overall and by study validation type. Assay performance based on the POR was positively associated with overall study quality (P=.015) and journal impact factor (P=.020). However, strong evidence for publication bias was observed based on both regression intercept (P<.001) and rank impact factor (P=.005). No significant differences in assay performance were noted for assays originally developed for response prediction (POR=5.3), prognosis (POR=6.6) or molecular classification (POR=6.9) (P=.770).

<table>
<thead>
<tr>
<th>Criteria</th>
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<th>Cell-Patient</th>
<th>External</th>
<th>Split Sample</th>
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<td>c-statistic</td>
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<td>40.9%</td>
<td>36.8%</td>
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<td>62.1%</td>
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<tr>
<td>Unfavorable Response Group</td>
<td>12.9%</td>
<td>17.1%</td>
<td>10.0%</td>
<td>12.0%</td>
<td>10.6%</td>
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</table>

Conclusions: While assay performance in predicting response to neoadjuvant chemotherapy based on multigene classifiers is encouraging, a compelling need exists for greater methodologic rigor and standardization of reporting. The predictive performance of multigene assay signatures varies with the type of validation sample utilized with external validation providing the most conservative estimates. No differences were seen for assays developed for prediction, prognosis or molecular classification. Considerable evidence for publication bias exists reflecting a paucity of smaller negative studies. The clinical validity of genomic response prediction assays should be evaluated in patient cohorts independent of those utilized for signature development. The clinical utility of these assays must then be further assessed in comparative effectiveness studies compared to commonly utilized clinical and laboratory measures.

Funding: NCI: UC2CA14041-01

**P5-13-18**

A Study of the Usefulness of Tumor Markers CA 15-3 and TPS in Monitoring of Different Subgroups of Metastatic Breast Cancer. Lindman H, Al-Musawi S, Nilsson G, Yachnin J. Uppsala University, Uppsala, Sweden

Background: The sensitivity and specificity of the tumor markers cancerantigen 15-3 (CA15-3) and the more seldom used tissue polypeptide specific antigen (TPS) for detecting and monitoring metastatic breast cancer are relatively well known. However, most of the studies in this area were performed before the era of breast cancer subtyping and some recent reports have showed differences in the sensitivity of CA 15-3 among different subtypes of breast cancer. This implies that the usefulness of a tumor marker could vary among different subgroups. We performed a retrospective study to find out the usefulness of CA 15-3 and TPS in long-term monitoring of metastatic breast cancer subgrouped by hormonal and HER2-receptor status. Patients and methods: CA 15-3 was routinely assessed regularly in all patients with metastatic breast cancer since 2003, while TPS was
assessed during years 2005 - 2009. In total 142 patients (median age 60 years, range 22-87) with metastatic breast cancer were identified in the local data base and included. The total number of analyzed CA 15-3 samples were 4232 (mean 30/patient) and TPS 1993 (mean 14/patient). In each patient the results of the markers were compared with disease response and progression during different lines of therapy and the patients were grouped based on the ratio of correct tumor marker values: “1” (useless) if 0-49% of the values were regarded as true, “2” (poor) if 50-74% were true, “3” (good) if 75-94% were true and finally “4” (excellent) if 95-100% of the values were considered true. If a patient had no elevated values during follow-up, she was reported as tumor marker negative: “0”.

Results: In general, the usefulness of CA 15-3 was excellent (4) in 50% of patients and good (3) in 16% more, compared with only 12% and 23% for TPS. Twenty-three percent and 25%, respectively were marker negative (0). There was a marked difference in the different subgroups. CA 15-3 was very useful (group 3 + 4) in estrogen and progesterone receptor positive patients (79%) and hormonal positive HER2 positive patients (82%), compared with only 40% in the triple negative group and 27% of the patients in the hormonal negative HER2 positive group. TPS showed the opposite tendency with less value in hormonal positive patients (only 31% in group 3 and 4) compared with 40% in triple negative and 45% the hormonal negative HER2 positive group. Other factors with strong positive influence of the value of the marker were high absolute values, increased value at diagnosis of metastatic disease (initial value) and visceral metastases.

Conclusion: Metastatic breast cancer can be well monitored using CA 15-3 in about 80% of the cases if the tumor is estrogen or progesterone positive. Hormonal receptor negative disease may be well monitored with CA 15-3 and/or TPS if there are elevated initial values, high absolute values and visceral disease. HER2-status seems not to influence the usefulness of CA 15-3 and TPS.

P5-13-19
Borderline Estrogen and Progesterone Receptor Expression and Efficacy of Anti-Estrogen Therapy Analyzed by Subpopulation Treatment Effect Pattern Plot Analysis.
Luok S-W, Ramey B, Park B, Keenan E. Oregon Health and Science University, Portland, OR; Portland Veterans Administration Medical Center, Portland, OR

Background: The American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP) recently recommended that estrogen receptor (ER) and progesterone receptor (PR) values be considered positive in the evaluation of breast cancer specimens if high absolute values and visceral disease. HER2-status seems not to influence the usefulness of CA 15-3 and TPS.

Methods: We examined a total of 608 patient samples from the OHSU Knight Cancer Institute Breast Cancer Tissue Repository for which we have quantitative ER and PR information and their clinical outcome. Of those, 282 patients did not receive any systemic treatment and 165 have quantitative ER and PR information and their clinical outcome.

Results: Multi-variate analysis finds neither ER nor PR expression adjusted by treatment status is associated with either overall survival (P=0.20, median follow up of 63 months) or breast cancer free interval (P= 0.82, median follow up of 60 months). For both ER and PR, there is no interaction between treatment outcome (5 year survival probability) and levels of hormone receptor expression based on Kaplan-Meier estimate (P=0.36 and P= 0.65, respectively). Patients with borderline ER expression appear to benefit from anti-estrogen therapy while patients with borderline PR expression appear to have worse outcome with anti-estrogen therapy.

Conclusions: Though descriptive in nature, our STEPP analysis provides support for the recommendation by ASCO and CAP to lower the cutoff for ER positivity in breast cancer specimens. There is overall no interaction between the level of hormone receptor and treatment benefit for all patients from low ER to high ER. Therefore, treatment will benefit all patients including those with low ER levels. Extending hormone therapies to the group of patients whose tumors express borderline PR values, however does not seem to confer therapeutic benefit. More study is needed to determine the benefits of offering anti-estrogen therapy to patients whose tumors express low PR.

P5-13-20
TOP2A Amplification Have Associated with Response to Anthracycline-Based Preoperative Chemotherapy in Primary Breast Cancer.
Wang J, Xu B, Yuan P, Zhang P, Li Q, Ma F, Zhao L. Cancer Hospital, Chinese Academy of Medical Science, Beijing, China

Background: Recent studies have suggested that HER2 gene amplification or overexpression have association with sensitivity of anthracycline-based chemotherapy. Preclinical studies found that TOP2A amplification or deletion only could be detected in HER2 amplification or over-expressive cases. And TOP2A protein is the target of anthracycline. In fact, the value of HER2 for predicting response to anthracycline-based chemotherapy in breast cancer may be more likely related to the concomitant amplification of the TOP2A gene. In this study we studied the association between TOP2A gene amplification and response to anthracycline-based preoperative chemotherapy. Methods: 309 early and local advanced breast cancer cases were enrolled in our study. HER2 proteins were qualitatively analysed by IHC, and TOP2A gene alterations were quantified by real-time polymerase chain reaction (RT-PCR) in primary tumor core biopsies from all HER2 over expressive cases. The enrolled patients received an intense dose dense (IDD) (CE, Cyclophosphamide + Epirubicin) or conventionally (TE, Paclitaxel+Epirubicin) scheduled anthracycline- based preoperative chemotherapy. The tumor was evaluated every two cycles. Median time on study for 309 patients with follow-up was 38 months. Results: The pCR was 14.3%. HER2 overexpression was found in 80/309 (25.9%) breast cancer cases, of which 61/80 cases have been tested for TOP2A status. 19/80 cases have not been tested for TOP2A status because of lacking core biopsies tumor tissue after pathological diagnosis. HER2 overexpression was associated with a significantly higher pCR rate compared to HER2 lower expression (27.5% vs. 9.6%, P=0.001). Further analysis was carried on and found the significantly higher pCR rate in TOP2A co-amplified cases compared to TOP2A deleted or normal cases.
(56.3% vs. 13.8%, P=0.001). HER2 overexpression was associated with a significantly higher pathologic complete response (pCR) rate only when TOP2A was co-amplified (56.3% vs. 9.6%, P=0.001), but not when deleted or normal (13.8% vs. 9.6%, P=0.183), compared to HER2 lower expression tumors. The interaction between HER2 or TOP2A and anthracycline-based regimen was observed not only in IDD but conventionally scheduled preoperative chemotherapy.

Discussion: Previous studies demonstrate that TOP2A gene amplification may define a subtype of HER2-positive breast cancer, this subtype of breast cancer may be highly sensitive to anthracycline-based chemotherapy, which may improve progression in HER2 and TOP2A co-amplification cases. However, this viewpoint needs to support from preoperative chemotherapy. Using RT-PCR to test TOP2A status from 61 primary breast cancer tumor tissue, we demonstrate that TOP2A amplification breast cancer have highly sensitive to anthracycline-based preoperative chemotherapy. This finding suggests that TOP2A as a predictive marker in breast cancer should be included in future studies.

P5-13-21
Japanese Patients with Discordance in Estrogen Receptor between Primary Breast Cancer and Recurrent Tumor Have a Poorer Outcome.
Tanaka K, Kawaguchi H, Kuba S, Koga C, Nishimura S, Yoshiyama T, Ishida M, Nakamura Y, Ohno S. National Kyushu Cancer Center, Fukuoka, Japan

Background
Decisions of systemic treatment for recurrent breast cancer are usually based on the estrogen receptor (ER), progesterone receptor (PR), and HER2 receptor status of the primary tumor. Recently, discordance in receptor status between primary and recurrent tumors has been reported. The objective of this study was to compare receptor status between the primary and recurrent tumor, and to evaluate overall survival (OS) after the diagnosis of recurrence between patients with discordant and concordant receptor status.

Material and Methods
Patients with recurrent breast cancer treated at National Kyushu Cancer Center between January 2004 and May 2011 were reviewed retrospectively. Primary and recurrent lesions were analyzed for ER and PR by immunohistochemistry (IHC) and HER2 by IHC or fluorescence in situ hybridization (FISH). OS in discordant and concordant groups for each receptor was estimated by Kaplan-Meier method and compared by log-rank test.

Results
Among 283 recurrent breast cancer patients both primary and recurrent tumor information for ER, PR, and HER2 was available in 69. Among these, recurrent biopsy specimens were obtained from locoregional recurrence in 72.5% and from distant metastases in 27.5%. Discordant rate in receptor status between the primary and recurrent tumor was 36.2%. Discordant rate in ER, PR, and HER2 was 14.4%, 23.1%, and 4.3%, respectively. Two-year OS after the diagnosis of recurrence in discordant and concordant ER group was 66.7% and 84.7%, respectively. The discordant ER group had significantly worse OS than the concordant ER group (P = 0.040). There was no significant difference in OS between the discordant and concordant PR group (P = 0.950).

Conclusions
Because discordance of receptor status between primary and recurrent tumors may exist, tissue confirmation of receptor status of recurrent tumor should be considered. Japanese patients with discordant ER between primary and recurrent tumor had a poorer outcome.

P5-13-22
Gene Expression Profiles Predict Pathological Complete Response to Standard Neoadjuvant Fluorouracil, Doxorubicin, and Cyclophosphamide and Paclitaxel with or without Trastuzumab in Early Breast Cancer.
Tamura K. National Cancer Center Hospital, Tokyo, Japan

Background: To examine the feasibility of gene expression signature as a predictor of pathological complete response (pCR) to sequential fluorouracil, doxorubicin, and cyclophosphamide (FEC) and weekly paclitaxel (P) with or without trastuzumab (T) neoadjuvant chemotherapy.

Materials and Methods: We have conducted consecutive two phase II, establishing training and validation sets, with similar eligible criteria include, stage IIA-IIIC, chemotherapy-naive, measurable disease, age ≥ 20, PS 0/1, and adequate organ function. Patients were treated preoperatively with 4 cycles of FEC (500/100/500 mg/m²) followed by 12 cycles of weekly P (80 mg/m²) with or without T (2mg/kg). Patients underwent pretreatment fine-needle biopsy for cDNA microarray using Affimetrix Gene Chip U133 plus 2.0 arrays with 30,000 differential expressions of various genes. We ranked gene probes from training sets according to a predictive power concerning pCR by Wilcoxon, and validated them using validation sets by SVM.

Results: Between July 2007 and December 2010, 122 patients were enrolled in the two consecutive prospective studies (training: 89 pts, validation: 33 pts). Median age was 51. PS 0/1: 115/7; Stage IIA/IIB/IIC: 30/57/20/14/1; Histological subtype: ER+HER2− (LA) / ER+HER2+ (LB) / ER-HER2− (TN) / ER-HER2+ (enrich-HER): 51/18/24/29. All patients have received curable operations. pCR rate was 31.1% (LA: 2.0%, LB: 44.4%, TN: 37.5%, enrich-HER: 69.0%).

104 (85.2%) sufficient mRNA for cDNA microarray from individual primary breast cancer tissues fine-needle biopsy are available. As reported previously, the breast cancers were classified into a Luminal A/B, Basal-like, HER2-enriched, Claudin-low intrinsic subtypes, indicating a high quality of the representative method. In HER2 positive breast cancer, HER2-enriched subtype was a reproducive predictive marker. In contrast, In HER2 negative breast cancer, three genes (N-myc and STAT interactor, Tryptophanyl-tRNA synthetase, and IQCE) and basal-like subtype were validated as the predictors of pCR. The three genes were also identified as predictors of pCR in the triple negative population.

Conclusions: Specific gene expression profiles predict pCR to standard neoadjuvant regimen, especially in triple negative breast cancer.

P5-13-23
Individualized Treatment Strategies for HER2-Negative Breast Cancer Subtypes.
Ishikawa T, Shimizu D, Yamada A, Sasaki T, Morita S, Tanabe M, Kawachi K, Nizawa A, Chishima T, Kimura M, Ichikawa Y, Endo I. Yokohama City University Medical Center, Yokohama, Kanagawa, Japan; Yokohama City University, Yokohama, Kanagawa, Japan

Background: Human epidermal growth factor receptor (HER2)-negative breast cancers are more heterogeneous than HER2-positive cancers, and tailored treatment is therefore required for luminal A and triple-negative breast cancer subtypes (LABC and TNBC). We therefore examined predictive factors for the efficacy of standard chemotherapy in LABC and TNBC subtypes.

Methods: A total of 109 LABC and 61 TNBC patients were treated with standard neoadjuvant chemotherapy (NAC) consisting of an anthracycline and/or taxane. The pathological treatment response
and prognosis were examined for each subtype. Expression levels of the following factors were examined in association with quasi-pathological complete response (QpCR): estrogen- and progesterone-receptor (ER and PgR) status, HER2, nuclear grade, MIB-1, p53, topoisomerase IIα (topoIIα), cytokeratin (CK) 5/6 and epidermal growth factor (EGFR).

**Results** QpCR rates in LABC and TNBC were 9.1% (10/109) and 54.1% (33/61), respectively. In LABC, the expression of PgR tended to be inversely associated with pathological response (p=0.087), while in TNBC, increased expression of topoIIα (p=0.006) and MIB-1 (p=0.018) were identified as predictors of QpCR. TopoIIα expression was also significantly associated with pathological response in multivariate analysis (p=0.014). The QpCR rate was higher in TNBC lacking CK5/6 and/or EGFR expression, defined as non-basal subtype (p=0.053).

**Conclusions** Low expression of PgR may be a possible predictor of the efficacy of chemotherapy in LABC, while a high level of proliferative activity, indicated by topoIIα and MIB-1, is associated with chemosensitivity in TNBC. Further subclassification into basal- and non basal-subtypes may also be helpful for the development of individualized treatments.

**P5-13-24**

**A Predictive Model of Early Systemic Disease Relapse after Standard Adjuvant Therapy for Breast Cancer.**


**Background:** Early relapse after adjuvant therapy for breast cancer is very discouraging and remains a major problem. We sought to identify predictors of early relapse risk and build a predictive model for relapse using prospectively collected data for patients seen at the Markey Cancer Center starting 2007 to date.

**Methods:** Of the 1098 new patients seen, 814 patients had stage I-III disease and were further analyzed for predictors of early relapse risk. Univariate analyses were performed for key variables including patient age, tumor size, grade, estrogen receptor (ER) status, progesterone receptor (PgR) status, and HER2 status. A multivariate Cox regression model was built to identify predictors of systemic relapse and model-building was performed using step-wise model selection to determine candidate models. A risk score was developed based on the linear combination of covariates in the final Cox model. Time-dependent predictive curves, a newly developed statistical methodology, were used to evaluate the predictive accuracy of the proposed risk score.

**Results:** Median patient age was 57 years (Range 25-92) and 88% were white. Forty six (46%) had stage I disease, 36% stage II, and 18% stage III. Median follow up time was 2.3 years. Of this 814 patient cohort, 706 patients had complete baseline covariate data and were used to build the candidate models. The final Cox regression model included 5 covariates that were significantly associated with risk of early relapse: stage III disease (p = 0.0011), grade III (p = 0.0028), PgR-negative status (p = 0.0121), HER2-negative status (p = 0.0305), and node-positive status (p = 0.0360). These five covariates were then used to calculate an early recurrence risk score, which is the weighted average of these risk factors when present, with the weights being the coefficients from the Cox regression model. The 1-year, 2-year and 3-year predictive curves for this risk score decrease considerably, especially for the 2-year and 3-year curves, indicating good predictive accuracy of the risk score. The highest risk score group, which represents 4.8% of the population, has a 1-year, 2-year and 3-year relapse probabilities of 13.0% (95% CI: 4.1%, 27.3%), 39.4 % (95% CI: 20.1%, 58.3%), and 52.3% (95% CI: 28.5%, 71.5%), respectively. In comparison, for the overall population, the corresponding 1-year, 2-year, and 3-year relapse probabilities were only 1.1% (95% CI: 0.5%, 2.1%), 4.2% (95% CI: 2.7%, 6.1%) and 6.2% (95% CI: 4.2%, 8.6%), respectively.

**Conclusions:** The developed risk score based on stage, tumor grade, PgR, HER2, and node status is highly predictive of early relapse in breast cancer patients after standard adjuvant therapy. Our model can be used to identify patients with high risk of early disease relapse who may otherwise benefit from enrollment on novel adjuvant therapeutic trials to improve their outcome.

**P5-14-01**

**Differences in Efficacy by Assessment Method: NCIC CTG Adjuvant Breast Cancer Trials MA.5, MA.12, MA.14, MA.21, MA.27 Meta-Analysis.**

Dong B, Chapman J-AW, Yerushalmi R, Goss PE, Pollak MN, Burnell MJ, Bramwell VH, Levine MN, Fritchard KJ, Whelan TJ, Ingle JN, Parulekar W, Shepherd LE, Gelmon KA. NCIC Clinical Trials Group, Queen’s University, Kingston, ON, Canada; Vancouver Cancer Centre-BCCA, Vancouver, BC, Canada; Harvard Medical School, Boston, MA; McGill University, Montreal, QC, Canada; Atlantic Health Sciences Corporation, Saint John, NB, Canada; Alberta Cancer Board, Calgary, AB, Canada; McMaster University, Hamilton, ON, Canada; University of Toronto, Toronto, ON, Canada; Mayo Clinic, Rochester, MN

**Background:** Based on recent breast cancer literature, we hypothesized that there could be substantive differences in apparent efficacy estimates using a log-normal (LN) survival model rather than with standard Kaplan-Meier (K-M) or Cox model methods. While both Cox and LN survival analyses offer greater specificity by individual patient characteristics, the LN model may more robustly estimate survival under model misspecification. Methods: We recently pooled data for 5 NCIC CTG primary breast cancer trials: MA.5, MA.12, MA.14, MA.21, and MA.27. The total patient count for patients who received at least 1 dose of trial therapy is 11,253. Compilation included definition of STEEP endpoints (C Hudis, JCO, 2008) and standardized factor categorizations. The primary endpoint is Breast Cancer Free Interval (BCFI) defined as the time from randomization until recurrence: first local invasive or DCIS; regional, or distant; contralateral invasive or DCIS; or death from breast cancer. We found substantive evidence of non-proportionality for 7 factors compiled for the meta-analyses. In this work, we fit multivariate Cox and LN models with these 7 factors, lymph node status and pathologic T status. We then compare BCFI efficacy estimates for patient and tumour characteristics at 1-, 3-, and 5-years obtained with K-M, Cox, and LN models. Results: There was evidence that the Cox assumption of proportional hazards was violated for 7 factors: age, menopausal status, hormone receptor status, anthracycline use, chemotherapy use, race, and ECOG performance status. Differences between models were intrinsically affected by timing and extent of non-proportionality; there was no consistent pattern. In particular, investigations to date indicate efficacy estimates with absolute differences between K-M, Cox and LN estimates which varied by time of assessment: at 1-year 0.0 to 6.7%, at 3-years 0.4 to 18.6%, and at 5-years 0.2 to 17.0%. BCFI estimates with the K-M were inconsistently closer to those with the LN or Cox model: for K-M to Cox at 1-year 0.4 to 5.2%, at 3-years 0.4 to 15%, at 5-years 0.4 to 14.3%; for K-M to LN at 1-year 0.0 to 6.7%, at 3-years 0.5 to 18.6%, at 5-years 0.2 to 17.0%; for Cox to LN at 1-year 0.8 to 1.8%.
at 3-years 1.9 to 6.0%, at 5-years 0.6 to 5.7%. K-M and Cox models have step-wise adjustments at events for K-M and Cox, rather than smooth modeling with the LN. Discussion: Even with reasonably large population subgroups, there were substantive differences in apparent survival (0.0 to 18.6%) between K-M, Cox and LN model types. The magnitude of differences in survival estimates was large enough to be clinically relevant and warrant further consideration as we evaluate new therapies and prognostic/predictive factors. We will be statistically investigating framework robustness under differing levels of model misspecification.

P5-14-02
Clinicopathologic and Prognostic Difference of Screen Detected Breast Cancer Compared with Symptomatic Breast Cancer.
Kim J, Lee SK, Kim S, Koo MY, Choi M-Y, Cho DH, Bae SY, Lee J, Jung SP, Lee JE, Yang J-H, Nam SJ. Samsung Medical Center, Seoul, Korea; Konkuk University Medical Center, Seoul, Korea
Background: Breast cancer screening program makes it possible to detect early cancer, thus to reduce breast cancer mortality. The authors studied clinicopathologic characteristics and prognosis of screen detected invasive breast cancer compared with symptomatic breast cancer. Furthermore, we compared the result according to molecular subtypes (luminal A, luminal B, Her2, triple negative), so intended to identify the role of screening in each subtypes.
Material and Methods: From January 2002 to June 2008 , 3141 patients who underwent operation for the treatment of invasive ductal carcinoma (NOS) at Samsung medical center were included. Among them, 1025 patient were screen detected, 2116 patient were symptomatic, out of screening over 2 years. We reviewed the medical records retrospectively.
Result: Screen detected breast cancer was associated with older patients, smaller tumor size, more hormone receptor- positive, less lymph node involvement, lower stage and reduced mortality compared with symptomatic breast cancer (P < .001). According to the molecular subtype, in luminal A subtype, the result shows better pathologic feature and also favorable overall and recur-free survival significantly.

Effect of screen detection on survival (screen detected vs symptomatic)

<table>
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<td></td>
<td>HR (95%CI)</td>
<td>p-value</td>
</tr>
<tr>
<td></td>
<td>HR (95%CI)</td>
<td>p-value</td>
</tr>
<tr>
<td>Total</td>
<td>1.55 (0.95 to 2.54)</td>
<td>0.095</td>
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<tr>
<td>Luminal A</td>
<td>2.31 (1.29 to 4.29)</td>
<td>0.018</td>
</tr>
<tr>
<td>Luminal B</td>
<td>1.49 (0.28 to 7.99)</td>
<td>0.641</td>
</tr>
<tr>
<td>Her2+</td>
<td>0.39 (0.14 to 1.07)</td>
<td>0.390</td>
</tr>
<tr>
<td>TNBC</td>
<td>1.17 (0.50 to 2.71)</td>
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</table>

HR : hazard ratio, TNBC : triple negative breast cancer

Conclusion: Compared to symptomatic breast cancer patients, screen detected breast cancer patients have favorable pathological and molecular characteristics, so better outcomes. According to the molecular subtype, only in luminal A subtype, screen detected breast cancer shows both overall and disease free survival benefit, and also acts as an independent prognostic factor itself. So, screening program seems to have a different efficacy depending on the molecular subtype of breast cancer

P5-14-03
Boobol SK, Kirstein L, Harshan M, Klein P, Cohen J-M, Chadha M, Baehner FL, Malamud SC. Beth Israel Medical Center, New York, NY; Genomic Health Inc., Redwood City, CA
Background: NCCN guidelines now include consideration of the 21 gene RT-PCR assay in node negative, hormone sensitive breast cancers greater than 0.6 cm. Recent data suggests a potential role for testing of node positive(N1), hormone sensitive patients as well. Currently, no data exists on testing of metastatic lymph nodes. We sought to establish the feasibility of Oncotype DX® testing in the metastatic lymph nodes and, furthermore, to evaluate the genomic concordance between the primary tumor and the nodal deposit. These results may further our understanding of tumor heterogeneity and biological selectivity in the process of lymph node metastasis and ultimately systemic metastases.
Methods: We examined the formalin fixed paraffin embedded tumor tissues (FPET) from 100 breast cancer patients from our institution’s pathology database with available paired primary tumor and macrometastatic nodal deposits by Oncotype Dx® testing. Inclusion criteria included patients with macrometastatic lymph node disease, hormone receptor positive, HER2 negative primary breast cancer. The testing laboratory was blinded from the clinical outcomes data available on these patients. All FPET samples had H&E slides made which were reviewed by board certified surgical breast pathologists to determine if there was sufficient invasive tumor and to direct dissection of all lymph node samples. The Recurrence Scores and quantitative single gene values from the paired samples will be examined descriptively with scatterplots and Pearson correlation coefficients and reported at the meeting.
Results: All 100 paired specimens were sent for Oncotype DX testing. 24 samples were found during standard H&E review to not have sufficient tumor for the assay. Of the 176 samples created for RNA extraction 173 samples had sufficient RNA for the Oncotype DX assay: 85 lymph node and 88 primary breast carcinoma samples. Recurrence Scores and quantitative single gene values from the paired primary breast carcinoma and lymph node samples will be examined descriptively and reported at the meeting.
Conclusion: Previous comparisons of paired primary and metastatic samples have used immunohistochemistry and FISH which are susceptible to variability in preanalytic variability such as delay to fixation, choice of fixative and duration in fixative. This study, using quantitative RT-PCR, will be one of the largest comparisons of tumor biology in paired samples yet reported in the era of genomic subtyping and may have implications for systemic adjuvant treatment.

P5-14-04
RANK Expression in Primary Tumor Tissue at the Time of Diagnosis Correlates with Risk of Subsequent Bone Metastases in the I-SPY 1 Trial (CALGB 150007/150012; ACRIN 6657).
Li J, Moore D, Yau C, Campbell M, Park J, I-SPY-1 TRIAL Investigator, Rugo HS. University of California, San Francisco, CA; Cancer and Developmental Therapeutics Program, Buck Institute for Age Research, Novato, CA
Background: RANK (receptor activator of nuclear factor kappa B) is a tumor necrosis factor receptor family protein that is expressed on the surface of osteoclasts and critical to bone turnover. RANK is also expressed on breast cancer (BC) cells, especially in the metastatic
setting. Pre-clinical mouse models have shown that the ligand to RANK (RANKL) can induce migration of RANK-expressed tumor cells to bone and lung, and that treatment with a RANKL inhibitor can reduce BC metastases. ISPY is a multicenter neoadjuvant study with well annotated gene expression data. We evaluated RANK expression in primary tumor tissues at the time of diagnosis (dx) in patients (pts) with BC and correlated expression with risk of subsequent metastases. Methods: We evaluated expression of genes in the RANK/RANKL pathway in core biopsies at dx, and correlated this data with risk of recurrence as well as risk of site specific metastases using the student t-test. Results: A total 221 pts were enrolled in this clinical trial, all had locally advanced BC (LABC) and received neo-adjuvant chemotherapy (96.6% pts received adriamycin + cyclophosphamide-based regimens), tumor samples were collected at the time of dx by core biopsy, and underwent gene array study. Total 38508 genes were tested and 149 pts had available gene array data. Average age was 48.0 yr, pt characteristics are listed in the Table. Analysis of variance (ANOVA) was then used to adjust clinical variability, after adjusting the difference of ER status, pathology subtypes, and menopausal status, RANK expression is even more highly expressed in the ER- group than ER+ group (p=0.006) and between the 2 groups. In addition, RANK was also significantly more expressed of RANK (p=0.05) in primary tumor tissues than those in primary tumor tissues at the time of diagnosis (dx) in patients (pts) who developed BDM had a significantly higher (p=0.07). When comparing gene expression in the RANK/RANKL pathway, pts who developed BDM had a significantly higher expression of RANK (p=0.05) in primary tumor tissues than those with NBDM, while there was no difference in RANKL expression between the 2 groups. In addition, RANK was also significantly more highly expressed in the ER- group than ER+ group (p=0.006) and in the basal group compared to the luminal A/B group (p= 0.002). Analysis of variance (ANOVA) was then used to adjust clinical variability, after adjusting the difference of ER status, pathology subtypes, and menopausal status, RANK expression is even more significantly correlated with the bone-dominant group (p=0.002).

Conclusion: Higher RANK expression in primary tumor tissues correlates with increased risk of subsequent BDM in pts enrolled in iSPY-1 trial. The sample size is small (n=149) and all pts had LABC. Our study suggests that targeting RANK/RANKL pathway is a promising strategy in preventing BDM in pts with high risk BC, further analysis in larger data sets is ongoing.

PS-14-05

Anti-Müllerian Hormone (AMH) Levels in Premenopausal Breast Cancer Patients Treated with Adjuvant Chemotherapy – A Translational Research Project of the SUCCESS Study.

Neugebauer JK, Rack BK, Kupka M, Dinkel C, Schneeweiss A, Schrader I, Tesch H, Rezai M, Söling U, Friese K, Beckmann MW, Janni W, Müller V. Ludwig-Maximilians-Universität, Munich, Germany; University Hospital Heidelberg, Heidelberg, Germany; Henriettestiftung Hannover; Hannover, Germany; Fachpraxis für Onkologie, Frankfurt, Germany; Luisenkrankenhaus Dusseldorf, Dusseldorf, Germany; Gemeinschaftspraxis Siehl & Söling, Kassel, Germany; University Hospital Erlangen, Erlangen, Germany; Heinrich-Heine-Universität, Dusseldorf, Germany; University Medical Center, Hamburg-Eppendorf, Germany

Background: Premenopausal women undergoing chemotherapy are at risk of premature ovarian failure and long term side-effects caused by premature menopause. However, knowledge about the rate of ovarian failure and potential markers to evaluate the ovarian reserve is limited, especially in the context of modern chemotherapy concepts. Therefore, Anti-Müllerian hormone (AMH) was measured at before, immediately after and 2 years after chemotherapy in premenopausal patients of the SUCCESS study.

Materials and Methods: The German SUCCESS trial is a multicenter phase III study comparing FEC-Docetaxel vs. FEC-Docetaxel+Gemcitabine as adjuvant treatment in patients with node positive or high risk node negative primary breast cancer. Blood samples were taken prior to and 4 weeks after last cycle of adjuvant chemotherapy, as well as after 2 years of follow up. We retrospectively identified 170 patients stratified premenopausal and aged 40 years or younger at trial entry, who received 3cycles of FEC (500/100/500ng/m2) q3w followed by 3 cycles of docetaxel (100mg/m2) q3w as one of the most commonly used chemotherapy regimens in Europe. Serum AMH levels were evaluated in a central laboratory by a manual immunoassay AMH DSL ELISA (Diagnostic Systems Laboratories, Webster, USA).

Results: Median age within this subgroup was 36 years (21-40 years). 48% of the patients had a tumor stage pT1 and 54% were node positive. 69% were hormone receptor positive and 29% Her2 positive. Median serum AMH level before adjuvant chemotherapy was 1.32 ng/ml (range <0.1-11.32). Immediately after chemotherapy AMH levels dropped in 96% of the patients below the threshold of detection (<0.1 ng/ml, range <0.1-3.9 ng/ml). Immediately after chemotherapy AMH levels dropped in 96% of the patients below the threshold of detection (<0.1 ng/ml, range <0.1-3.9 ng/ml). No association to classical prognostic markers, such as tumor stage, lymph node involvement, etc. was observed. After a follow up period of 2 years, serum was available from 95 patients. 76% of those patients showed no evidence of ovarian function indicated by AMH (<0.1 ng/ml, range <0.1-1.43 ng/ml). AMH levels prior to and 2 years after chemotherapy were significantly correlated with older age, with a reduction of 0.14 ng/ml per life year (p=0.0025) and 0.01 ng/ml (p=0.017) respectively. 12 patients (7%) received optional gonadotropin-releasing hormone (GnRH) agonists during chemotherapy. These patients presented significantly higher AMH levels (+ 0.18 ng/ml; p=0.01) 2 years after cytotoxic treatment. Conclusion: In this retrospective analysis premenopausal patients showed a high rate of ovarian insufficiency reflected by low serum AMH levels immediately after cytotoxic treatment and after 2 years of follow up. GnRH agonists given as ovarian protectants during chemotherapy may have an influence on serum AMH 2 years after chemotherapy. Further data from prospective trials with longer follow up are needed to evaluate the role of serum AMH as a predictor of ovarian failure in breast cancer patients exposed to chemotherapy.
P5-14-06
Interaction between Stoma and Tumor Characteristics as a New Prognostic and Predictive Marker in Breast Carcinomas.
Tagliabue E, Sandri M, Casalin P, Aiello P, Pupa SM, Orlandi R, Balsari A, Triulzi T. Fondazione IRCCS- Istituto Nazionale dei Tumori, Milan, Italy; University of Milan, Milan, Italy
Background. We recently demonstrated that primary breast tumors can be classified based upon extracellular matrix composition (ECM) (Bergamaschi et al.) suggesting stroma characteristics could influence tumor progression. Aim of this study is to further investigate the robustness of this classification and its impact in tumor progression and response to therapy.
Methods. The expression profile of ECM-related genes was analyzed by unsupervised hierarchical clustering in 10 independent datasets of breast tumors, counting more than 1000 samples and the prognostic and predictive value of this signature were evaluated in two datasets of not treated patients and in one dataset of neo-adjuvant treated patients, respectively.
Results. Only one of ECM subsets (ECM3) showed a homogenous gene pattern that consistently allowed the classification of an independent group of tumors in all tested datasets. ECM3 is characterized by highly correlated over expression of 34 ECM genes encoding mainly structural proteins. From 24 to 38% of cases were ECM3-enriched and were mainly estrogen receptor-positive and low grade (p<0.0001). The ECM3 showed inconsistent association with DMFS in the two data sets of not treated patients, whereas the interaction between ECM3 and grade showed strongly and significant association with DMFS (HR=5.35, p=0.0012). In particular, multivariate analysis of covariates available (size, ER, age and ECM) indicated that ECM3 is significantly associated with worse DMFS in grade III patients (HR=2.5, p=0.0386), whereas it is slightly correlated with better DMFS in grade I-II patients (HR=0.6, p=0.088). It is noteworthy that the probability of 10-year DMFS was 90% in ECM3 versus 74% in non-ECM3 patients in differentiated tumors, and 75% in non-ECM3 versus 41% in ECM3 grade III tumors.
Conclusions. Our results provide evidence that breast carcinoma progression and response to therapy are influenced by the interaction between tumor and stromal characteristics. (Partially supported by AIRC).

P5-14-07
Novel Interactions between Immunity and Proliferation in Breast Cancer Progression.
Nagalla S, Chou JW, Ruiz J, Vaughan JP, Black MA, Miller LD. Section on Hematology and Oncology; Department of Biostatistical Sciences; Wake Forest University Health Sciences, Winston-Salem, NC; Otago School of Medical Sciences, University of Otago, Dunedin, New Zealand
Highly proliferative breast tumors are phenotypically and clinically heterogeneous and exhibit a significant metastatic tendency. However, not all highly proliferative breast cancers will progress to distant metastasis, suggesting that other factors may play a role in limiting their clinical progression. With an interest in identifying genes that predict metastatic capacity of breast cancers in a proliferation-dependent context, we recently compiled a database of ~2,000 tumor expression profiles using microarray data from multiple large breast cancer cohorts. We randomized the dataset into training and testing sets to allow discovery and validation of novel gene-survival (ie, distant metastasis-free survival; DMFS) associations. We employed Cox regression analysis to identify genes with statistically significant associations with DMFS. Then, we hierarchically clustered the expression patterns of the significant genes to identify gene clusters lacking correlation with proliferation genes. A large cluster of genes with known functions in innate and adaptive immunity emerged. Cross referencing these genes to a published microarray dataset of pan-leukocyte expression profiles revealed cell type-specific structure within the immunity cluster, likely reflective of distinct populations of tumor-infiltrating immune cells. Specifically, 3 immune gene subclusters identified within the tumors showed predominant expression in B-cells, T-cells and natural killer (T/NK) cells, or monocytes and dendritic (M/D) cells. To evaluate the prognostic relevance of these immune gene clusters, we averaged the gene expression measurements within each cluster to generate “metagenes” that could be used to divide breast cancer cases into population tertiles based on the low, intermediate and high expression of the immune genes. The same was done for a large cluster of proliferation genes known to reflect the proliferative capacity of breast cancer cells. Strikingly, we found that that prognostic power of the B-cell, T/NK and M/D metagenes was not consistent among the proliferation tertiles, but rather, was exclusively statistically significant in the highest proliferation tertile, enriched for basal-like, luminal B and HER2+-like tumors. In these tumors, all 3 immune metagenes stratified cases into low, intermediate and high risk of recurrence with high statistical significance (p<0.0001), wherein the highest expression tertile of the immune metagenes corresponded to reduced risk of metastatic recurrence (at 5 and 10 years), consistent with a long-term anti-metastatic immune response. Comparative analysis of the B and T/NK metagenes revealed a highly significant prognostic interaction between the two, whereby the low expression tertile of either the B-cell or T/NK metagene portends a poor prognosis that is not overcome by a high expression tertile of the other. Our results suggest that cell type-specific immune signatures in breast cancer can predict survival in highly proliferative subtypes of breast cancer, with potential to spare some patients from aggressive adjuvant treatment while identifying others who will require more than standard of care. Furthermore, our results provide novel insight into the important roles of T and B cell-mediated immunity in breast cancer progression.

P5-14-08
Impact of Progesterone Receptor Semiquantitative Immunohistochemical Result on Oncotype DX® Recurrence Score: A Quality Assurance Study of 1078 Cases.
Bhargava R, Dabbs DJ. Magee-Womens Hospital of UPMC, Pittsburgh, PA
Background: The oncoype DX® recurrence score (RS) is predominantly dependent on estrogen and progesterone receptor content of the tumor, proliferation and HER2 status. We have previously shown (Mod Pathol. 2008;21:1255-1261) that using a regression equation that incorporates the above along with tumor grade components is capable of estimating the RS score. In the present study, we tested this formula in 1078 prospectively collected breast cancer cases.
Methods: We employed a logistic regression equation that incorporates the above along with tumor grade components to estimate the RS score. Using this formula, we assigned an RS score to the tumors. We then compared this calculated RS score with the RS score derived from the Dimethylaminoazobenzene modified avidin-biotin immunoperoxidase method. We also noted clinical outcome of the patient.
Results: We assigned an RS score of 18 to 25% of cases and an RS score of 26 to 30% to 39% of cases. There was no significant difference in the calculated RS score and the RS score derived from the staining method. Furthermore, we observed no correlation of RS score with the type of surgery, tumor size, nodal status, lymphovascular invasion, and hormone receptor status.
Conclusions: Our data suggest that the RS can be estimated accurately and reliably by using a formula that incorporates the above factors and tumor grade components.
Results: A total of 1078 cases were analyzed (see table below).

<table>
<thead>
<tr>
<th>Tumor stage</th>
<th>PR score: median, mean (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade I and low RS (n=185)</td>
<td>10; 172 (159-185)</td>
</tr>
<tr>
<td>Grade I and intermediate RS (n=150)</td>
<td>150; 138 (120-156)</td>
</tr>
<tr>
<td>Grade I and high RS (n=75)</td>
<td>75; 134 (13-134)</td>
</tr>
<tr>
<td>Grade II and low RS (n=287)</td>
<td>287; 150 (149-151)</td>
</tr>
<tr>
<td>Grade II and intermediate RS (n=247)</td>
<td>247; 150 (149-151)</td>
</tr>
<tr>
<td>Grade II and high RS (n=287)</td>
<td>287; 150 (149-151)</td>
</tr>
<tr>
<td>Grade III and low RS (n=29)</td>
<td>29; 200 (149-211)</td>
</tr>
<tr>
<td>Grade III and intermediate RS (n=66)</td>
<td>66; 130 (113-158)</td>
</tr>
<tr>
<td>Grade III and high RS (n=74)</td>
<td>74; 76 (58-94)</td>
</tr>
</tbody>
</table>

RS: recurrence score; CI: confidence interval; IHC: immunohistochemical

Conclusions: Within each grade, PR IHC score is inversely related to the RS. The dramatic impact of PR IHC score on RS is noteworthy. If PR IHC score is combined with traditional histopathologic features (nuclear grade, mitotic activity score), and other IHC variables (ER IHC score, HER2 status, Ki-67 labeling index), then a tumor’s risk of recurrence and need for chemotherapy can be reasonably estimated. OncoType DX® test may not be necessary in more than half of cases that are currently sent for testing.

P5-14-09
Malignant Calcification Is an Important Prognostic Factor in Women with Invasive Breast Cancer – Suggestion for Regarding It as High-Risk Factor in Current Staging and Adjuvant Treatment Guidelines.
Ling H, Shao Z-M, Liu Z-B, Xu X-L, Yang W-T, Xu L-H, Gu Y-J. Fudan University Shanghai Cancer Center, Shanghai, China

Purpose:
To investigate whether calcification is a prognostic factor for breast cancer.

Methods:
A total of 804 patients with invasive breast cancer diagnosed between 2001 and 2003 were recruited and divided into two groups based on whether they had malignant calcification on mammogram. The association of calcification with lymph node status, grade, tumor size, ER, PR and Her2 status, 8-year disease-free survival (DFS) and overall survival (OS) was evaluated. Survival difference was analyzed by Kaplan-Meier technique. The relative importance of each of the potentially prognostic variables was tested using Cox regression analysis.

Results:
The incidence of malignant calcification in all breast cancer was 41.5%. Compared with tumor without calcification, those with calcification had a larger size (2.6cm vs 2.1cm, P=0.000), higher axillary lymph node (LN) positive rate (40.1% vs 31.5%, P=0.012), more LN involvement (14.8% vs 9.2%, P=0.000), lower ER (54.3% vs 65.1%, P=0.002) and PR (42.7% vs 60.6%, P=0.000) expression and higher Her2 expression (27.3% vs 14.7%, P=0.000).
The 8-year DFS was lower for patients with calcification than those without (89.9% vs. 76.8%, P=0.000). The 8-year OS for tumor with calcification was 82.2%, compared with 92.8% for those without (P=0.000).

Subgroup analysis showed that the shorter survival and higher relapse rate for tumors with calcification could be found in both LN- and LN+ patients, also could be found in T1, T2, Grade II, ER+, PR+ and Her2- patients.
The existence of calcification, LN stage, tumor size, ER status, and grade were significantly associated with survival in univariate analysis. Further Cox regression analysis demonstrated that calcification and LN stage maintained their prognostic significance in both DFS and OS. The hazard ratios of these factors are listed in Table 1.

<table>
<thead>
<tr>
<th>Hazard Ratios from Multivariate Cox Regression Analysis to DFS &amp; OS</th>
<th>DFS</th>
<th>OS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcification</td>
<td>P=0.001</td>
<td>P=0.001</td>
</tr>
<tr>
<td>sex</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>yes</td>
<td>1.90</td>
<td>2.16</td>
</tr>
<tr>
<td>LN stage</td>
<td>P=0.000</td>
<td>P=0.000</td>
</tr>
<tr>
<td>no</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>N1</td>
<td>1.75</td>
<td>2.12</td>
</tr>
<tr>
<td>N2</td>
<td>1.57</td>
<td>1.98</td>
</tr>
<tr>
<td>N3</td>
<td>0.61</td>
<td>1.15</td>
</tr>
<tr>
<td>Tumor Size</td>
<td>P=0.330</td>
<td>P=0.911</td>
</tr>
<tr>
<td>ER</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>PR</td>
<td>P=0.570</td>
<td>P=0.240</td>
</tr>
<tr>
<td>HER-2</td>
<td>P=0.020</td>
<td>P=0.040</td>
</tr>
<tr>
<td>yes</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>no</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Calcification</td>
<td>P=0.083</td>
<td>P=0.111</td>
</tr>
<tr>
<td>Grade</td>
<td>P=0.320</td>
<td>P=0.320</td>
</tr>
<tr>
<td>Grade I</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Grade II*</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Grade III</td>
<td>1.25</td>
<td>1.48</td>
</tr>
</tbody>
</table>

*Univariate analysis shows there’s no significant difference of survival between Grade I and II

Conclusions:
The existence of calcification is a poor prognostic factor for patients with invasive breast cancer. Its prognostic value is only second to LN status and is higher than the other factors evaluated, including tumor size, grade, and ER, PR status. Thus, breast cancer with calcification should be regarded as a high-risk factor when determining cancer stage and selecting the adjuvant treatment.

Discussion:
Given the strong prognostic value of calcification revealed in this research, the biological mechanism of its formation and related cell signal pathway should be investigated. Further adjuvant clinical trial should be done to select certain sensitive chemotherapeutic agent or regimen. Some biomarkers have been found to have a role in the formation of calcification and may be targets for treatment.

P5-14-10
Ethnic Differences in the Association between Tumor Size and Lymph Node Status among Breast Cancer Patients in South East Asia.
Saxena N, Verkooijen HM, Bhoo Pathy N, Siew EL, Jau P, Lee SC, Yip CH, Hartman M. National University of Singapore, Singapore, Singapore; University Medical Center Utrecht, Utrecht, Netherlands; Julius Center University of Malay, Kuala Lumpur, Malaysia; National University Cancer Institute, National University Health Systems, Singapore, Singapore; Faculty of Medicine, University of Malaya Medical Center, Kuala Lumpur, Malaysia

Background: South East Asia consists of three major ethnic groups, i.e. Chinese, Malays and Indians. Previous work from our breast cancer research group showed that Malay ethnicity is an independent
predictor of poor survival. In this study we evaluate whether (part of) these ethnic survival disparities may be due to ethnic differences in the association of tumor size and lymph node involvement.

Methods: We included all patients diagnosed with breast cancer at the National University Hospital (Singapore) and University of Malaya Medical Center (Kuala Lumpur) between 1990 and 2007, for whom information on tumor size and axillary lymph node status was available (n = 3805). Ethnic differences in the association between tumor size and lymph node involvement were studied and logistic regression analysis was performed to determine the independent effect of ethnicity on the association of tumor size and lymph node involvement.

Results: 1474 patients were categorized as T1 (>0 to 2 cm), 1777 as T2 (2.1 to 5 cm) and 554 as T3 (>5 cm). Within the small tumor size range (>0 to 2 cm) the Malays were significantly more likely to present with node positive disease as compared to the other ethnic groups (36.9% as compared to 24.5% for Chinese and 25.9% for Indians, p-value <0.001). Similarly, for tumors measuring 2.1-5 cm, Malays were more likely than Chinese and Indians to have lymph node involvement (59.5%, 50.2% and 52.1%, respectively, p-value 0.016). After adjustment for age, Progesterone Receptor (PR) status, grade and treatment received, Malay ethnicity was an independent predictor of lymph node involvement, with adjusted Odds Ratio’s of 1.4 (95% CI 1.0 to 2.1) for T1 tumors and 1.4 (95% CI 1.1 to 1.9) for T2 tumors compared to Chinese women.

Conclusion: Malay patients are more likely to present with lymph node metastasis especially for the small and midsized tumors as compared to the Chinese and Indians. This could reflect differences in tumor biology between the three ethnic groups with the Malays possibly having more aggressive disease than the others.

P5-14-11
Elevated Serum Ferritin Predicts Reduced Progression-Free and Overall Survival in Trastuzumab-Treated Metastatic Breast Cancer.
Alkhateeb AA, Connor J, Leitzel K, Ali S, Campbell-Baird C, Evans M, Koestler W, Fuchs E-M, Lipton A. The Pennsylvania State University Hershey Medical Center, Hershey, PA; Lebanon VA Medical Center, Lebanon, PA; Medical University of Vienna, Vienna, Austria

Background: Approximately one-half of HER2-positive breast cancer patients will not respond to first-line trastuzumab-containing therapy. Since trastuzumab is now used in the HER2-positive adjuvant breast cancer setting, trastuzumab resistance will continue to be a vexing clinical problem, and better predictive and prognostic biomarkers are urgently needed.

One potential biomarker is serum ferritin. Although serum ferritin has been used as an indicator of total body iron, a specific functional role has not been proposed thus far other than perhaps recycling iron for erythropoiesis. Increased serum ferritin levels have been reported in breast cancer patients. However, the prevailing paradigm on ferritin at the time of these observations did not encourage speculation on its role or significance and was dismissed as a non-specific parameter for cellular damage. There are now multiple lines of evidence that have challenged this traditional paradigm and suggested ferritin to be a multi-functional factor involved in key cellular and systemic processes including immune regulation, angiogenesis, and iron delivery.

Methods: Pretreatment serum ferritin was measured using an ELISA assay in 66 metastatic breast cancer patients before starting first-line trastuzumab-containing therapy. Serum ferritin was determined using an ELISA from Assaypro, St. Charles, MO. Progression-free survival (PFS) and overall survival (OS) were analyzed using the Kaplan-Meier method and Cox modeling, with separate analyses as continuous serum ferritin, or as dichotomous categorical groups using the median pretreatment serum ferritin level as a cut off point. To interrogate a functional impact of elevated serum ferritin, we used a cell culture model.

Results: When analyzed as dichotomous categorical groups using the median pretreatment serum ferritin level as a cut off point, the elevated serum ferritin patient cohort had a significantly reduced OS (P<0.0001, median OS 12.73 vs. 69.57 months) and PFS (P=0.004, 8.30 vs. 23.90 months). In the cell culture model, ferritin bound to breast cancer cells promoted proliferation and activated AKT signaling. These novel observations suggest that the iron storage protein ferritin has a signaling role in tumor biology. Since the elevation in serum ferritin is unlikely a consequence of a change in total body iron, we also examined several possible sources for this elevation. Macrophages, but not breast cancer cells, were capable of ferritin secretion.

Conclusions: Elevated serum ferritin predicts reduced PFS and overall survival in metastatic breast cancer patients treated with first-line trastuzumab-containing therapy. The ability of ferritin to activate AKT signaling could underlie the trastuzumab resistance in patients. Therefore, serum ferritin may not only have predictive value as a clinical tool but also has direct functional significance in the treatment response, progression and survival of breast cancer patients.

P5-14-12
Bayesian Belief Network Mortality Analysis of a Breast Cancer Registry Data Set.
Eberhardt JS, Hyslop T, Mitchell E, Hu H, Rui H. DecisionQ Corporation, Washington, DC; Thomas Jefferson University, Philadelphia, PA; Windber Research Institute, Windber, PA

Introduction: Bayesian Belief Networks have been used in medicine to evaluate clinical data and develop predictive and prognostic models. As classification models, they allow us to represent pattern complexity beyond what can be accomplished with traditional Kaplan-Meier or regression models. We sought to evaluate the use of machine-learned Bayesian Belief Networks (ml-BBNs) to develop mortality models in breast cancer and to evaluate classification performance for this method.

Methods: A set of 2,300 breast cancer cases from a tumor registry at Thomas Jefferson University were used to train ml-BBNs. The registry set was broken into cohorts for modeling by follow-up times of 1 (n=2,202), 2 (n=2,183), 3 (n=2,157), and 5 (n=2,027) years. Each cohort was then used to train a ml-BBN and each model was evaluated for structure. Variables were recoded into categories: biomarkers (ER, PR, Ki67, HER2, p53) as positive or negative; grading, staging, and size were broken in categories; while race was recoded into Caucasian or African-American. Income and poverty level by census tract were also included. Models were evaluated for ability to classify mortality (yes/no) within the follow-up period using 10-fold cross-validation and Receiver Operating Characteristic curves.

Results: Area Under the Curve (AUC), Positive Predictive Value (PPV), and Negative Predictive Value (NPV) were calculated for each set of cohort training models and mean values and 95% confidence intervals were calculated for mortality (yes/no) within the follow-up period. AUCs (and CIs) for 1, 2, 3, and 5 years were: 0.81 (0.70 – 0.91), 0.74 (0.69 – 0.79), 0.81 (0.77 – 0.86), 0.77 (0.74 – 0.80). PPVs for 1, 2, 3, and 5 years were: 12.3% (7.5% – 17.1%), 18.8% (15.4% – 22.1%), 18.0% (15.1% – 20.9%), 28.2% (24.7% – 31.7%). NPVs for 1, 2, 3, and 5 years were: 99.2% (98.8% – 99.7%), 97.4% (96.9% – 97.8%), 96.4% (95.1% – 97.7%), 91.7% (89.1% – 94.3%). Predictors
of mortality at 1 year were Tumor Stage, at 2 years were Estrogen Receptor and Tumor Stage, and at 3 and 5 years were Diagnosis Age, Tumor Stage, Estrogen Receptor status, and Ki-67 receptor status.

Discussion / Conclusion: We were able to successfully train mLBNNs to estimate mortality using breast cancer registry cohorts. Cross-validation showed the models to be robust. The structure of the models can inform us how different data elements contribute to the estimate of mortality. These models can be used to calculate individual probabilities for prognostic guidance given age, staging criteria, and biomarkers. Overall 5-year mortality in the study set is 15.2%, however we can derive subject-specific mortality estimates. For example, a 43-year old Stage 3, ER-Negative, Ki-67 Negative subject has a 19.9% probability of 5-year mortality, while the same subject with positive Ki-67 has a 37.8% probability of mortality. Meanwhile, the same probabilities for a 70 year old woman are 67.0% and 59.0%, respectively.

P5-14-13
Favorable Prognosis in Patients with T1a,b Node-Negative Triple Negative Breast Cancers Treated with Multimodality Therapy.
Ho AY, Gupta G, Perez CA, King TA, Patil SM, Rogers KH, Brogi E, Morrow M, Hudis C, Traina T, McCormick B, Powell SN, Robson ME. Memorial Sloan Kettering Cancer Center, New York, NY

Purpose: To evaluate the clinical characteristics, natural history and outcomes in patients with ≤1 cm, node-negative triple negative breast cancer (TNBC).

Materials and Methods: After excluding patients who received neoadjuvant therapy, 1,022 TNBC patients who received definitive breast surgery from 1999 to 2006 were identified from an institutional database. Among these, 194 patients had node-negative tumors ≤1 cm and comprise the study population. Clinical data was abstracted and survival outcomes were analyzed.

Results: Median follow-up time was 71 months (range 2-143). Median age at diagnosis was 55.5 years (range 27-84). T stage was T1mic in 16 (8.2%), T1a in 49 (25.3%), T1b in 129 (66.5%). The majority of tumors were poorly differentiated (N=142,73%), lacked lymphovascular invasion (N=170,87.6%) and were screening-detected (69%, N=134). Breast-conserving surgery (BCS) was employed in 129 (66.5%) and mastectomy in 65 (33.5%) patients. 113 (58%) patients received adjuvant chemotherapy and 123 (63%) received whole breast radiation. Patients who received chemotherapy tended to have more adverse clinical and disease features (younger age,T1b, poor tumor grade; all p<0.05). For the entire group, 5 year local recurrence-free survival was 96% and distant metastasis-free survival was 95%, with no difference in distant relapse rates between T1mic/T1a vs. T1b patients (94.5% vs 95.5%, p=0.81) or by receipt of chemotherapy (95.9% vs 94.5%, p=0.63).

Conclusion: Excellent 5-year locoregional and distant control rates were achievable in patients with TNBC tumors ≤1.0 cm, 58% of whom received chemotherapy. These results identify a group of TNBC patients with favorable outcomes following early detection and multimodality treatment.

P5-14-14
Van den Eynden GG, Van Laere SJ, Smid M, Martens JW, Foevens JA, Vermeulen PB, Dirix LY. Augustinus Hospital, Antwerp, Belgium; Erasmus Medical Center Rotterdam, Rotterdam, Netherlands

Introduction: The fibrotic focus (FF) is a practical, easily assessable and reproducible integrative histological prognostic parameter in breast cancer. Its prognostic value has been shown before (Van den Eynden et al. Histopathology 2007). In this study we investigated whether the assessment of the FF adds prognostic information to the relapse score based on gene expression analysis of 76 genes as previously described (Wang et al. Lancet 2005).

Materials and Methods: All patients of 2 previous prognostic breast cancer gene expression studies for whom FFPE slides of the tumor were available (Wang et al. Lancet 2005 and Yu et al. BMC Cancer 2007) were selected, leading to a study population of 176 lymph node negative breast cancer patients. The presence and size (<1/3 or >1/3 of tumor area) of a FF were assessed on standard HE slides. These data were compared to the 76-gene relapse score, to standard clinicopathological variables and to metastasis-free survival.

Results: A small and large FF were found in 31 (17.6%) and 20 (11.4%) of patients, respectively. In 120 (68.2%) patients there was no FF and in 5 patients the presence of a FF could not be assessed due to insufficient FFPE material. 64 (36.4%) and 112 (63.6%) patients had respectively a good and poor prognostic 76-gene relapse score. There was a significant correlation between the presence of a FF and a poor 76-gene relapse score, 18 of 20 patients with a large FF had a poor relapse score (p = 0.03). Patients with a tumor with a FF and especially with a large FF had a significantly reduced metastasis-free survival (Log rank p<0.001). The same was true for patients with a poor 76-gene relapse score (Log rank p<0.001). When only patients with a poor relapse score were taken into account, patients with a tumor with a large FF had a significantly decreased metastasis-free survival compared to patients without a FF or with a small FF (Log rank p<0.005). In patients with a good relapse score, the number of patients with a FF was too small for a separate analysis. In a multivariate Cox regression model for metastasis-free survival including age, ER and PR status, T stage, the FF and the 76-gene relapse score status, the FF (OR 1.5, p=0.02) and the relapse score (OR 2.0, p=0.001) were independent prognostic factors. Comparable results were found if the presence of a FF was dichotomized in large FF versus no or a small FF.

Conclusion: The assessment of the presence and size of a FF adds independent significant prognostic information to the prognostic 76-gene expression signature, especially in selecting a subgroup of patients with a very poor prognosis. Since the assessment of the FF is practical, easy, reproducible and cheap it should be considered to become part of the standard pathological examination of breast cancer resection specimens.
P5-14-15
Prognostic Impact of Chemotherapy-Induced Amenorrhea (CIA) in Premenopausal Breast Cancer: A Meta-Analysis of Published Literatures.
Lu J, Zhou Q, Yin W. Fudan University Shanghai Cancer Center, Shanghai, China

Background: Various studies have reported inconsistent results on the prognostic effect of chemotherapy-induced amenorrhea (CIA) in premenopausal breast cancer. Therefore, we conducted a meta-analysis to assess the effect of CIA on prognosis of premenopausal women with breast cancer.

Methods: The PubMed database was searched for all relevant studies published before March 2011. Relative risks (RRs) were used to estimate the association between CIA on various survival outcomes. Subgroup analyses were also performed by age, nodal status and hormone receptor (HR) status.

Results: With thirteen eligible studies identified, this meta-analysis included 5790 cases and 2159 controls. This meta-analysis demonstrated that CIA was associated with improved DFS (RR=0.39, 95%CI: 0.32-0.47, p<0.001) and OS (RR=0.30, 95%CI: 0.21-0.44, p=0.001). In the subgroup analyses, the effect of CIA on DFS and OS also existed in HR-positive subjects (for OS, RR=0.19, 95%CI: 0.07-0.49, p=0.001; for DFS, RR=0.47, 95%CI: 0.35-0.63, p=0.001) while similar results failed to be observed in HR-negative patients (for OS, RR=0.62, 95%CI: 0.07-5.75, p=0.671; for DFS, RR=1.48, 95%CI: 0.50-4.37, p=0.474). Furthermore, significant difference was achieved between women with and without CIA, irrespective of age and nodal status.

Conclusion: This meta-analysis clarifies that CIA might contribute to the improvement of prognosis in HR-positive premenopausal breast cancer patients, which is at least partially responsible for the benefit of adjuvant chemotherapy in premenopausal women through chemical castration.

P5-14-16
Molecular Classification in Primary Breast Cancer and Corresponding Lymph Node Metastasis Show Impaired Prognostic Profile in the Metastatic Node.
Falck A-K, Ferno M, Bendahl P-O, Rydén L. Clinical Sciences, Lund, Sweden

Introduction
The heterogeneity of breast cancer disease has emphasized the need to optimize prediction of outcome by molecular classification of the primary tumor. Clinical management of primary breast cancer is based on biomarkers measured in the primary tumor, whereas biomarker analysis in metastatic lymph nodes is not used for clinical decision making. Recently, microarray-based expression profiling and subsequent immunohistochemical studies have identified biologically distinct subtypes using a combination of biomarkers. The present study aimed to classify primary breast cancer tumors and corresponding lymph node metastases by multiple biomarkers into Luminal A, Luminal B, HER2 type and Triple negative phenotype and compare outcome.

Material and method
The study is based on a cohort of patients where biomarker expression (hormone receptor status (ER, PR), human epidermal growth factor receptor 2 (HER2) and Ki67) in primary breast cancer and ipsilateral lymph node metastasis recently were reexamined and individually related to prognosis. In the present study, 85 patients of the original cohort were possible to classify into the four subtypes: Luminal A (ER+, PR+/- and Ki67 ≤ 20%), Luminal B (ER+, PR+/-, HER2+/- and Ki67 > 20%), HER2- type (ER-, HER2+) and Triple negative (ER-, PR-, HER2-). The classifications of primary tumors and corresponding lymph node metastases were compared using the McNemar-Bowker test of symmetry and the molecular subgroups were related to clinical outcome in terms of 5-years distant disease-free survival (DDFS) by Cox analysis with luminal A as the reference group.

Results
In 7 cases (16%) the biomarker classification was discordant between the primary tumor and the lymph node metastasis. All of the discordant cases shifted to a subtype with a worse prognosis according to the lymph node metastasis (p=0.06, McNemar-Bowker test of symmetry). When comparing Luminal A to non-Luminal A in the primary tumor and the lymph node, the deviation from symmetry was significant, p=0.02. DFS was worse in all subtypes compared to the prognosis for Luminal A using Cox analysis in both primary tumors and lymph node metastasis.

Conclusion
Our data shows discordance in classification according to a multiple molecular phenotype between primary tumors and lymph node metastasis in breast cancer patients. The discordant cases were shifted from Luminal A to a subtype where survival analysis showed an impaired prognosis compared to this subgroup. The finding that the molecular phenotype is shifted from a “good” prognostic signature in the primary tumor to a “bad” prognostic signature in the metastatic node can be of clinical importance for the choice of adjuvant systemic therapy in primary breast cancer with lymph node positive disease.

P5-14-17
Stage IV at Presentation – Are HER2 Positive Tumors Overrepresented?

Background: A minority of patients are diagnosed with Stage IV breast cancer at presentation. Recent studies (Dawood 2010) have suggested a better outcome for de novo vs. recurrent Stage IV but they did not account for the variation of molecular subtype. We questioned whether HER2 overexpressing tumors were over-represented in de novo Stage IV disease, and whether this impacted on survival compared to other subtypes. Further, if different subtypes are more likely to present with metastatic disease, then this factor may need to be considered when developing guidelines for staging. With such considerations in mind, the purpose of this study was to determine the breast cancer subtypes according to stage. The main hypothesis was that HER2 positive tumors would be more prevalent in stage IV presentations.

Methods: Using the Breast Cancer Outcomes Unit database from the BC Cancer Agency (BCCA), patients referred to the BCCA with a new diagnosis of breast cancer between 2005 and 2010 were selected. Patients with a previous or synchronous contralateral breast cancer, male cases, and patients with referrals for reasons other than new disease were excluded. Four subtypes according to available markers were defined: ER+/HER2-, ER+/HER2+, ER-/HER2+, and ER-/HER2-.

Results: Using these criteria, 485 cases of de novo stage IV disease and 10,723 stages I - III cases were extracted. After excluding cases with missing data, our final cohort consisted of 10,186 stage I-III cases and 425 stage IV cases. Distribution by subtype is presented in the Table below.
Assessment of other patient characteristics for the group of Stage IV de novo patients revealed that age (younger for HER+ subgroups), site of metastases (more visceral vs. non-visceral for ER-/HER2+ and ER-/HER2-), and type of systemic therapy (chemotherapy (CT), hormone therapy (HT), trastuzumab (T) and not) were significant. Surgery rates for both mastectomy and breast-conserving surgery were similar for all subtypes. The ER-/HER2- subtype had the worst overall survival ($p < 0.001$).

### Conclusion

Young age and HER2 overexpression is more common in stage IV de novo presentations (26.6% of stage IV tumors were HER2+ vs. only 16% of stage I-III tumors). This data may be important in considering routine staging guidelines at diagnosis to ensure correct diagnosis and treatment recommendations.

## P5-14-18


Madsen EV, Elias SG, Gobardhan PD, van Oort PM, van der Ent FW, Nieweg OE, Valdés Olmos RA, Smidt M, van Dalen T. Diakonessenhuis Utrecht, Utrecht, Netherlands; University Medical Centre Utrecht, Utrecht, Netherlands; The Netherlands Cancer Institute - Antoni van Leeuwenhoek Hospital, Amsterdam, Netherlands; Maastricht University Medical Centre, Maastricht, Netherlands; Orbis Medical Centre, Sittard, Netherlands

Background: Her-2-neu receptor (Her2) positive and triple negative breast cancer patients have a poor prognosis. The majority of cancers are characterized as estrogen receptor (ER)+/Her2- and these patients may now have a better prognosis compared to before the introduction of the Her-2-neu receptor.

Material and methods: Since 1997 3424 patients were treated for cT1-2N0 breast cancer in three hospitals. Determination of Her2-neu status was introduced between 1999 and 2004. Trastuzumab treatment has been given routinely since 2005. Survival was evaluated for the different groups: ER+/Her2-, ER+/Her2+, ER+/Her2 unknown (status not determined).

Results: 2284 patients had ER+/Her2- tumors, 259 had ER+/Her2+ tumors and 262 had ER+/Her2 unknown tumors. Systemic treatment was given to 48.4%, 71.0% and 43.9% respectively. Estimated 5- and 10-year overall survival was 92.0% and 82.2% for ER+/Her2-, 91.6% and 70.8% for ER+/Her2+ and 83.4% and 72.2% for ER+/Her2 unknown ($p < 0.001$). The outcome differences between ER+/Her2- and ER+/Her2 unknown tumors remained following adjustment for tumor malignancy grade, nodal status and adjuvant systemic treatment (OR 0.8: CI 0.72 - 0.88; $p<0.001$). For patients with ER+/Her2 tumors 5 year overall survival was comparable with ER+/Her2+ tumors but 10 year overall survival was comparable with ER+/Her2 unknown tumors.

Discussion: Patients with ER+/Her2- tumors have a significantly better outcome than patients who were classified as ER+ before the assessment of Her-2-neu over-expression. Current prognostic models do not take this effect into account.

The branching off of the survival curve for patients with ER+/Her2+ tumors can be explained by the standard use of trastuzumab during the last five years.
and medical decision making, but prior to using whole Korean patients, this model requires validation with multiple Korean sources.

References

P5-14-20

Purpose of the study: In a new database harbouring the patients of several prospective phase II neoadjuvant trials (fec 50-100, net, tncf, taxotere/tncf, taxotere alone), the predictive value of tumour factors (SBR grade, RH, Ki-67 and cycline D1) was studied in 466 women with stage II-III operable breast cancer treated between 1991 and 2006.

Patients and methods: Median age of the patients was 48 years [27-76]. Median diameter of the invasive tumour was 40 mm [10-130]. 382 (82%) patients had a canalar, 57 (12.2%) a lobular, 14 (3%) a mixed or invasive carcinoma, 3 (0.6%) neoplastic cells only and 10 (2.2%) another carcinoma. Before chemotherapy, 33% were grade III SBR. The median number of NCT courses was 6 [1-8] followed by a surgery for 98%, a radiotherapy for 93%, an adjuvant chemotherapy (20%) and/or a hormonotherapy (56%).

Results: Overall response rate was 73% (18% complete). The complete pathological response (pCR) rate was 17.2% according to Chevallier’s classification. On 455 patients operated, 324 (71%) had a conservative surgery. On 390 patients with an axillary dissection, 195 (50%) had involved nodes (median number : 2 [1-20]). After a median follow-up of 135 months, DFS and actuarial survival at 120 months were 61.9% and 73%, respectively.

After chemotherapy, we found a significant variation for SBR grade: there was five fold more patients for whom we observed a down grading of the SBR grade (grade 2/3 to grade 1) than up grading (grade 1 to grade 2/3), p=6.1.10^-6. For patients in objective response, we observed a significant decrease of SBR grade (p=1.7.10^-8).

Moreover, the variation of SBR grade was also prognostic. Indeed, the disease-free survival for patients who presented a SBR down grading after chemotherapy was significantly better (p=0.031) compared to patients for whom SBR grade remained stable or was in up grading. There was a tendency for overall survival (p=0.15).

Conclusion: Down grading of SBR grade appears linked to response and so may constitute a parameter of better prognostic. At the opposite, up grading of SBR grade is an adverse prognostic factor. To conclude, it is interesting to emphasize that SBR grade variations can be assessed when patients are not in pCR, ie plays a complementary role.

P5-14-21

Purpose. Lymph node involvement is the most important prognostic factor in breast cancer. It is a multifactorial event determined by tumour and patient characteristics. Several predictors of axillary lymph node metastasis (ALNM) in breast cancer have been described. The purpose of this study was to determine clinical and pathological factors predictive for ALNM in patients with early breast cancer and to build a model to portend lymph node involvement.

Methods. We evaluated 1300 consecutive patients surgically treated in our institution (2007-2009) for cT1-T2 invasive breast cancer. The patient and tumour characteristics evaluated included: age at diagnosis, number of foci, histologic grade, location, tumour size, histologic subtype, lymphovascular invasion (LVI), estrogen-receptor (ER), progesterone-receptor (PR) and Her2 status. Univariate and multivariate analyses were performed. Factors significantly associated with lymph node metastasis by univariate analysis and histologic subtype were included in the multivariate model. We validated our model with the same 1300 patients on the basis of a multivariable logistic regression model containing the variables, using a correction factor.

Results. By univariate analysis, the incidence of ALNM was significantly higher in patients with a tumour with LVI (P < 0.0001), larger tumours (P < 0.0001), tumours with a higher histologic grade (P < 0.0001), tumours located retroareolar or lateral in the breast (P < 0.0001), tumours with multiple foci (P = 0.0002) and in patients who underwent an axillary lymph node dissection. We found no effect of ER/PR nor HER-2 status.

<table>
<thead>
<tr>
<th>Variable</th>
<th>OddsRatio (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.001 (0.992 - 1.010)</td>
<td>0.8259</td>
</tr>
<tr>
<td>Number of foci</td>
<td>1.398 (1.173 - 1.665)</td>
<td>0.0002</td>
</tr>
<tr>
<td>Histologic grade</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall effect</td>
<td></td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Tumour location</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall effect</td>
<td></td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Tumour size</td>
<td>1.050 (1.045 - 1.067)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Histologic subtype</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall effect</td>
<td></td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Lymphovascular invasion</td>
<td></td>
<td>0.5738</td>
</tr>
<tr>
<td>Present vs. absent</td>
<td>0.640 (0.419 - 1.15)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Estrogen-receptor status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive vs. negative</td>
<td>1.263 (0.928 - 1.719)</td>
<td>0.1362</td>
</tr>
<tr>
<td>Progesterone-receptor status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive vs. negative</td>
<td>1.044 (0.386 - 1.351)</td>
<td>0.7435</td>
</tr>
<tr>
<td>Her-2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive vs. negative</td>
<td>0.731 (0.383 - 2.923)</td>
<td>0.6584</td>
</tr>
<tr>
<td>Procedure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALNM vs. SNLB</td>
<td>2.940 (2.331 - 3.709)</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

By multivariate analysis, lymph node involvement was significantly associated with the presence of lymphovascular invasion (P < 0.0001), larger tumour size (P < 0.0001), axillary lymph node dissection (P = 0.0003), retroareolar and lateral tumour location in the breast (P = 0.0019) and the presence of multiple foci (P = 0.0155).
Results: The first 65 patients evaluated are presented. Disease stabilization beyond 3 years. The interim results from the randomized regimen who maintained a partial or complete remission or disease stabilization during at least 3 years. The preliminary findings support that trastuzumab provides a substantial long-term survival benefit with a manageable safety profile in HER2+ MBC patients. This study adds to the evidence that there may be benefit in continuing trastuzumab after achieving remission or disease stabilization. Final results will be presented in the forthcoming congress.

P5-14-22
Prospective Observational Study To Describe the Clinicopathological and Biological Characteristics and the Management of Metastatic Breast Cancer Patients Who Experienced Complete or Partial Remission or Disease Stabilization during at Least 3 Years.

Zamora P, Pérez-Carrión R, Manso I, Crespo C, Mendiola C, Álvarez-López I, Margeli M, Bayo-Calero JL, González-Farre X, Santaballana A, Ciruelos EM, Afonso R, Lao J, Catalán G, Álvarez-Gallego JV, Miramón-López J, Salvador-Boffil FJ, Ruiz-Borrego M, Hospital La Paz, Madrid, Spain; Hospital Quiron, Madrid, Spain; Hospital 12 de Octubre, Madrid, Spain; Hospital Ramón y Cajal, Madrid, Spain; Hospital Donostia, San Sebastián, Spain; Hospital Germans Trias i Pujol, Badalona, Spain; Hospital Juan Ramón Jiménez, Huelva, Spain; Hospital Clinic de Barcelona, Barcelona, Spain; Hospital La Fe, Valencia, Spain; Hospital Nuestra Señora de la Candelaria, Santa Cruz de Tenerife, Spain; Hospital Miguel Servet, Zaragoza, Spain; Hospital Son Llàtzer, Mallorca, Spain; Complejo Hospitalario de Zamora, Zamora, Spain; Hospital Serranía de Ronda, Ronda, Spain; Hospital Virgen de Valme, Sevilla, Spain; Hospital Virgen del Rocio, Sevilla, Spain

Background: Trastuzumab has shown an improvement in survival outcomes among patients with HER2+ metastatic breast cancer (MBC). Identification of pathological, clinical factors and tumor genetic profile that may predict long-term remission has become a key-issue. We aimed to describe the clinicopathological and biological characteristics of MBC patients who experienced complete response (CR), partial response (PR), or stable disease (SD) during at least 3 years and their management in routine clinical practice.

Methods: Multicenter, observational, cross-sectional study. Data were collected from women with HER2+ MBC treated with a trastuzumab-based regimen who maintained a partial or complete remission or disease stabilization beyond 3 years. The interim results from the first 65 patients evaluated are presented.

Results: Median age: 59 (52-70) years. Metastatic disease was diagnosed after a median of 23.5 (1.6-48.8) months since primary tumor diagnosis. The predominant tumor type was ductal carcinoma (89.2%) and 47% showed histological grade III. Mean tumor size: 3.6±2.2cm (anatomical pathology), 5.1±2.8cm (imaging studies). Hormonal status: Progesterone receptor positive 46% and estrogen receptor positive 43%. Most common metastatic sites: lung (23%), liver (17%) and bone (14%). Overexpression of HER2 was assessed by IHC in 97% of patients, of whom 94% were HER2+ (3+) and 17% had FISH+HER2 status. Tumor was positive for p53 and Ki67 in 23% and 41.5%, respectively. Surgery was performed on 83% of patients, of which 73% underwent radical mastectomy; 96% had their axillary nodes removed. Surgery of metastases was performed on 7.8%. First line chemotherapy was received by 91% with the most frequent schemes being paclitaxel (24%), vinorelbine (15%) and paclitaxel/carboplatine (14%). First line hormonal therapy and radiotherapy was used in 45% and 12%, respectively. All patients received first line trastuzumab, administered on a weekly schedule in 51%. Trastuzumab was used in combination in most of patients (89.2%) with a median number of cycles of 18 (7.0-41.5) and during a median of 53.3±25 months. 66% of patients achieved a CR, 21% PR and 13% had SD. Median time since trastuzumab was initiated to CR, PR or SD was 5 (4-7) months. Median duration of CR, PR or SD was 36 (44.5-78.0) months. Trastuzumab was maintained beyond CR, PR or SD in 99% during a median of 46.5 (35-67) months. 75% of patients continue on treatment with trastuzumab. Only 2 patients discontinued trastuzumab due to toxicity. At the time of the analysis, 19% had progressed, 57% were alive and free of disease and among patients on treatment (93%), 54% were on trastuzumab. Cardiac toxicity was the most common toxicity (36%) among those suffering at least one (22%).

Conclusions: The preliminary findings support that trastuzumab provides a substantial long-term survival benefit with a manageable safety profile in HER2+ MBC patients. This study adds to the evidence that there may be benefit in continuing trastuzumab after achieving remission or disease stabilization. Final results will be presented in the forthcoming congress.

P5-14-23
Clinical Outcomes of Different Subtypes Detected by Immunohistochemistry of Early Invasive Breast Cancers in a Monoinstitutional Series.

Ferro A, Ecccher C, Caulara A, Triolo R, Barbareschi M, Cuervo LV, Aldovini D, Dipasquale M, Galligioni E. S Chiara Hospital, Trento; FBK, Trento; S. Chiara Hospital, Trento

Background: Early invasive breast cancer (EIBC) is an heterogeneous disease. Immunohistochemical (IHC) markers can be used to classify tumors in different biological subtypes with different clinical behavior.

Purpose: The aim of our study was to evaluate survival outcomes for patients (pts) with different subtypes of EIBC as classified using four ICH markers (ER, PR, HER2 and Ki67).

Methods: We evaluated data from 3403 consecutive cases of EIBC treated from 1995 to 2008 and classified as: luminal A (positive ER and PR, negative HER2 and Ki67<14%), luminal B (positive ER and/or PR, negative HER2 and Ki67<14%), Luminal B (positive ER and/or PR, positive HER2, any Ki67), HER2+ (negative ER and PR, positive HER2, any ki67), triple negative (negative ER and PR, negative HER2, any ki67). Log-rank test and Kaplan-Meyer estimator were performed to evaluate the impact of ICH subtypes on overall survival (OS), Event Free Survival (EFS) and their correlation with other known prognostic factors such as N, G, Size, Age.

Results: We identified 917 luminal A (26.9%) , 1731 luminal B (50.9%), 279 (8.2%) luminal C, 183 HER2 + (5.4%) and 293 triple negative (8.6%). Median age was 61 years. Luminal A was more frequently (p<0.001) associated with older age, smaller size, negative axilla involvement, low grading. Observed events (relapses, contralateral and second tumors) were: 54 in luminal A (6%), 215 in luminal B (16%) , 40 in luminal C (14%), 42 in HER2+ (23%) and 59 in triple negative (20%). Disease free interval (DFI) was shorter in luminal C, HER2 and TN (median DFI: 30, 26 and 19 months) than in Luminal A and B (median DFI: 51 and 41 month). Luminal A and B presented more bony and less visceral recurrences than luminal C,
HER2 and triple negative tumors. At median follow up of 51 months EFS was 94.1% in luminal A, 87.5% in luminal B, 85.5% in luminal C, 76.8% in HER2+ and 79.7% in triple negative. Corresponding OS was 95.3% in luminal A, 89% in luminal B, 89.2% in luminal C, 80.9% in HER2+, 81.9% in triple negative. Different subtypes EFS according to nodal status, grading, tumor size and age, was reported in table 1.

In multivariate Cox-regression analysis correcting for tumor size, differentiation grade, lymph node status, hormone receptor status, tumor stage and treatment modalities (surgical procedure, chemotherapy, radiotherapy and antimetabolite therapy), the CK19 bone marrow status still was a significant predictor of IDFS. (p-value = 0.042)

Differences in BCSS (78% of CK19+ patients and 84% of CK19- patients) and OS (75% in CK 19+ patients and 74% in CK 19- patients) were not statistically significant. (log rank p-values 0.087 and 0.883, respectively).

**Conclusion:** This study demonstrates the long term (>ten years) prognostic effect of CK19 mRNA detection with RT-PCR in the bone marrow of operable breast cancer patients.

### P5-14-25

#### Disease Presentation, Treatment, and Outcome in Young and Elderly Women with Breast Cancer.

Yu E, Yu D, Godette K, Mister D, Torres M. Emory University, Atlanta, GA

**Aim:** To determine the influence of age on disease presentation, treatment, and outcome in breast cancer patients who are not routinely screened by mammography, women younger than 40 and women older than 69 years.

**Methods:** The records of 272 breast cancer patients who presented to the Emory University Department of Radiation Oncology between 1997 and 2010 were reviewed. We excluded women with inflammatory or Stage IV breast cancer and those diagnosed between the ages of 40 and 69. We compared presentation, staging, treatment, and outcomes in women younger than 40 with those older than 69.

**Results:** The median age of the young and older patient groups were 31 (range 16-40) and 76 years (range 70-91), respectively. Young women more often presented with a symptomatic breast mass than older women whose cancers were more frequently detected on screening mammography (p<.001). Young women were less likely than older women to present with significant co-morbidities including hypertension, diabetes, or cardiac disease (2% vs 76%, p<.001). A higher proportion of young women had high grade (50% vs 25%), T2/ T3 tumors (69% vs 26%), and node positive disease (50% vs 23%) (p<.001 for all comparisons) than older women. Also, more young women had triple negative (25% vs 12.5%, p<.048) or HER2 positive breast cancers (34.4% vs 18.3%, p=.02). Regarding treatment, older patients were less likely to undergo surgical axillary nodal staging (72% vs 88%, p<.04) or chemotherapy (23% vs 84%, p<.001). Among women treated with chemotherapy, older women more often received a non-anthracycline based regimen (82% vs 7% of young patients, p<.001). Following neoadjuvant chemotherapy, significantly more young women had a complete pathological response (23% vs 13% in older women, p<.03). Fifty-two percent of young women received mastectomy versus 14% of older women (p<.001). In part due to their low rates of breast conserving surgery, younger women trended toward less radiation treatment (81% vs 90% in older women, p=.12). Among estrogen receptor positive patients, more young women received adjuvant endocrine therapy than older women (91.4% vs 72%, p=.05). The median follow-up period for the entire group was 38 months (range 1-160). At 3 years, young women were more likely to recur locoregionally (10% vs 2%, p=.06) and distantly (16% vs 5%, p=.04) than older women, though both groups had comparable breast cancer-specific (96% vs 96%, p=0.2) and overall survival (96% vs 93%, p=.3). On multivariate analysis, old age was

### Table 1

<table>
<thead>
<tr>
<th>Subtypes EFS by N, G, size and age</th>
<th>Luminal A (%)</th>
<th>Luminal B (%)</th>
<th>Luminal C (%)</th>
<th>HER2+ (%)</th>
<th>Triple negative (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>94.1</td>
<td>87.5</td>
<td>85.5</td>
<td>76.8</td>
<td>79.7</td>
</tr>
<tr>
<td>N0</td>
<td>94.5</td>
<td>85.5</td>
<td>86.2</td>
<td>80.7</td>
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</tr>
<tr>
<td>N1</td>
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<td>G1</td>
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<td>83.3</td>
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<td>95.6</td>
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<td>96.3</td>
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<td>T1</td>
<td>95.6</td>
<td>91.7</td>
<td>86.7</td>
<td>81.6</td>
<td>86.8</td>
</tr>
<tr>
<td>T2</td>
<td>88.9</td>
<td>83.4</td>
<td>79.3</td>
<td>72.5</td>
<td>75.9</td>
</tr>
<tr>
<td>T3</td>
<td>91.3</td>
<td>73.2</td>
<td>76.5</td>
<td>72.7</td>
<td>55.2</td>
</tr>
<tr>
<td>Age &lt; 40</td>
<td>90.5</td>
<td>75.9</td>
<td>75.9</td>
<td>75.9</td>
<td>83.3</td>
</tr>
<tr>
<td>Age 40-55</td>
<td>91.8</td>
<td>88.4</td>
<td>83.5</td>
<td>79.6</td>
<td>76.3</td>
</tr>
<tr>
<td>Total</td>
<td>94.1</td>
<td>87.5</td>
<td>85.5</td>
<td>76.8</td>
<td>79.7</td>
</tr>
</tbody>
</table>

Considering only Luminal subtypes, Luminal B and C were significantly associated with poor EFS vs Luminal A EIBCs in both N0 (p=0.046) and N+ pts (p=0.001), in T1 (p=0.013) and T2 (p=0.03) pts, in pts older than 40 years (p=0.002).

**Conclusions:** Luminal A showed better prognosis in term of EFS and OS than other subtypes regardless other prognostic factors as clinical features (age) and tumor extent (T and N).
Conclusion: The results of our study suggest that Oncotype DX is applicable and feasible to perform in UK patients with a reduction in the use of adjuvant chemotherapy consistent with findings of reported studies. RS added prognostic information beyond information provided by Adjuvant!

**P5-14-26**

Results from a Prospective Clinical Study on the Impact of Oncotype DX on Adjuvant Treatment Decision Making in a Cohort of 142 UK Patients.

Holt S, Bertelli G, Brinkworth E, Durrani S, Jones S, Khawaja S, Laggner U, Moe M, Pudney D, Pitcher S, Rolles M, Sharaia Y, Whelan S, Prince Philip Hospital, Llanelli, Carmarthenshire, United Kingdom; Singleton Hospital, Swansea, West Glamorgan, United Kingdom; Bronglais Hospital, Aberystwyth, Ceredigion, United Kingdom

Objectives: International guidelines support the use of the Oncotype DX derived Recurrence Score (RS) to provide additional prognostic and predictive information in early breast cancer but experience in the UK is limited. In our prospective study we evaluate this test for the NHS and its impact on costs (subject of a separate abstract) and treatment recommendations by UK oncologists.

Methods: 150 tests were made available to consecutive patients with ER+, pN0, pN1ic or pN1mic early breast cancer who had no contraindication to adjuvant chemotherapy (CT) and who would accept CT + hormone therapy (HT) if recommended. CT recommendations of oncologists based on Adjuvant! Online figures were recorded at an initial consultation. Eligible patients were consented to Oncotype DX testing and review arranged once the result was available. After a second consultation a final decision on adjuvant therapy was recorded.

Results: Analysis is based on 142 patients. (150 tests performed, 3 failed to give a result, 3 repeated tests giving a result on the second block, one bilateral and one test stopped because the patient withdrew from the study). Initial treatment recommendations changed in 13 (26.8%) cases. Of the patients initially recommended CT + HT (total 57 patients), 26 (45.6%) patients were spared chemotherapy after review with the RS. Of the 85 patients initially recommended HT only 12 (14.1%) were changed to HT + CT.

Further analysis shows that Grade, estrogen receptor status by immunohistochemistry (ER by IHC) and progesterone receptor by immunohistochemistry (PR by IHC) are correlated to RS but in our cohort age, size and node status were not. (Spearman rank correlation for grade is 0.05, 95%, CI 0.36 to 0.61; for ER by IHC is -0.36, CI -0.49 to -0.20; and for PR by IHC is -0.49, 95%, CI -0.60 to -0.35). Apart from HER2 positive patients who are already recognized to return a high RS, further analysis of our series shows no clear combination of currently available prognostic factors that would predict RS and therefore reliably avoid testing of any subset of patients.

**P5-14-27**

Prognostic Factors of Node-Negative, High Risk and 1-3 Positive Lymph Nodes Breast Cancer by Intrinsic Subtype in Patients with Adjuvant Chemotherapy.

Kojima Y, Hashimoto K, Harano K, Shimizu C, Yunokawa M, Yonenori K, Tamura K, Katsumata N, Ando M, Kinoshita T, Fujiiwara Y. National Cancer Center Hospital, Tokyo, 5-1-1 Tsukiji, Chuo-Ku, Japan

Purpose: St. Gallen 2007 categorized high risk, node-negative breast cancer (HNBC) and 1-3 lymph nodes positive BC (LNBC) without HER2 overexpression as intermediate risk. We hypothesized that triple negative BC (TNBC) and hormone-receptor positive without HER2 overexpression (H-BC) in intermediate risk have different prognostic factor in patients with adjuvant chemotherapy (AdC).

Methods: We examined disease-free survival (DFS) and overall survival (OS) of TNBC and H-BC with regard to potential prognostic factors. All the patients included in this study were categorized intermediate risk by St.Gallen 2007 and received AdC.

Results: A total of 470 patients were identified; H-BC (n=360) and TNBC (n=110). Age (<35) was significantly associated with DFS in TNBC while it was not in H-BC (p=0.0.1 and p=0.63, respectively). Tumor size (>2cm) and tumor grade related to DFS in H-BC but not in TNBC. Tumor grade was not associated with DFS in both H-BC and TNBC (p=0.64 and p=0.91, respectively). Lymph node metastasis was a significant factor of DFS only for H-BC (p=0.009 and p=0.19). Conclusions: In TNBC, age was only a significant factor associated with DFS while in H-BC, lymph node status and tumor size rather age related to DFS.

**P5-15-01**

Words Matter: Influence of DCIS Diagnosis Terminology on Patient Treatment Decisions.

Omer Z, Hwang ES, Esserman LJ, Ozanne EM. University of California, San Francisco; Massachusetts General Hospital

Background: Treatment of ductal carcinoma in-situ (DCIS) poses significant challenges. Although retrospective studies suggest that most cases of low grade DCIS will never progress to invasive disease, it remains difficult to accurately identify those patients at greatest risk. In this situation where many diagnosed tumors could follow an indolent course for the patient’s lifetime, both systemic therapy and watchful waiting could be reasonable options, similar to those currently offered to patients with early prostate cancer. One strategy that has been suggested to reduce overtreatment is to use terms other than “ductal carcinoma in situ” when explaining a diagnosis of DCIS to a patient. In the current study, we have investigated the effect of terminology on women’s stated treatment preference for DCIS.

Methods: Women 40-65 years of age were recruited from a database of volunteers at an academic hospital. Subjects with a personal history of breast cancer were excluded. Endpoints were gathered from a web-based survey. Each subject was presented with three different scenarios, each of which used a different terminology for DCIS: non-invasive breast cancer, breast lesion, and abnormal cells. The scenarios included a detailed explanation of the risks and benefits of three treatment options: surgery, systemic treatment only and active surveillance. After reading each scenario, the subject was asked to...
choose among the treatment options and to explain her choice. Results: 187 subjects completed the survey. More women chose active surveillance when DCIS was described using the terms “abnormal cells” or “breast lesion” than using the term “non-invasive cancer” (Table 1). The majority of women (97/187, 52%) changed their treatment preference when a different term was used to describe DCIS. Of the 97 women who changed their treatment preference, 47 (48%) chose surgery when the term “non-invasive cancer” was used to describe DCIS, but chose a less invasive treatment when “cancer” was not used in the diagnosis. Of 90 people who did not change their treatment preference, 39 (43%) chose active surveillance.

Among the three treatments, the percent of women who chose surgery was the highest (84/187; 46%) when the term “non-invasive cancer” was used. 47/84 (56%) of the women who chose surgery when using the term “non-invasive breast cancer” changed their treatment preference when a different term was used. 36/84 (43%) switched to active surveillance while 11/84 (13%) switched to medication.

Conclusion: These results support that the specific terminology used to explain a diagnosis of DCIS influences patients' treatment preference. Moreover, we found that women may entertain treatment preferences other than surgery for DCIS when the tradeoffs of each choice are clearly explained. Avoiding the word “cancer” in the diagnosis may offer a strategy that reduces fear-based treatment decisions and may reduce the burden of overtreatment for DCIS.

<table>
<thead>
<tr>
<th>Participant choices:</th>
<th>Terminology used to describe the diagnosis</th>
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<tbody>
<tr>
<td></td>
<td>Non-invasive breast cancer Number (%)</td>
</tr>
<tr>
<td>Total (n=187)</td>
<td></td>
</tr>
<tr>
<td>Surgery</td>
<td>84 (46)</td>
</tr>
<tr>
<td>Medication</td>
<td>38 (21)</td>
</tr>
<tr>
<td>Active Surveillance</td>
<td>62 (33)</td>
</tr>
</tbody>
</table>

P5-15-02

Patients’ Views about How Oncologists Should Explain Prognosis in Advanced Cancer.

Kiely BE, McCaughan G, Christodoulou S, Tattersall MH, Beale P, Grimison P, Stockler MR. University of Sydney, Sydney, NSW, Australia; Royal Prince Alfred and Concord Hospitals, Sydney, NSW, Australia

Background: Many women with advanced breast cancer want information about their life expectancy. Understanding the type of information patients want will help oncologists improve their discussions about prognosis. We sought the attitudes of women previously diagnosed with breast cancer to using three scenarios for survival (best case, worst case and most likely) to explain prognosis to people with advanced cancer.

Methods: Members of the Breast Cancer Network Australia (BCNA) review and survey group and oncology clinic attendees with a previous breast cancer diagnosis were invited to complete a questionnaire describing two options for explaining prognosis to a hypothetical patient with incurable cancer who wanted prognostic information: providing either 3 scenarios for survival or just the median survival time. Associations between respondent attitudes and their demographic and tumour characteristics were explored.

Results: Characteristics of the 254 BCNA respondents and the 68 outpatient respondents respectively were: median age 56 years and 54 years; median years since breast cancer diagnosis 6 and 3; college/university educated 70% and 34%; self-report of cancer spread beyond the lymph nodes 10% and 26%. Significantly more respondents agreed that explaining 3 scenarios (vs just the median survival time) would: be easy to understand (92% v 71%), be helpful (94% v 63%), convey hope (68% v 36%), reassure (56% v 30%) and help family members (92% v 65%), with all p-values <0.001.

The proportions of respondents agreeing that each of the 3 scenarios should be presented were: best case 92%, worst case 84% and most likely 93%. Although 53% agreed the worst case scenario on its own was likely to upset people, only 31% agreed that explaining all 3 scenarios together was likely to upset people. When asked how they would prefer information about their own prognosis, 92% wanted all 3 scenarios, either with (49%) or without (43%) the estimated median survival. Most respondents (88%) agreed it would be helpful to receive a printed summary of the scenarios for survival. No differences were seen between the BCNA respondents and breast cancer outpatients for attitudes to presenting all 3 scenarios; however, BCNA respondents were significantly less likely than breast cancer outpatients to agree that the median survival was helpful, reassuring or easy to understand. Women with more education were more likely to agree that presenting all 3 scenarios would be helpful (95% v 87%, p = 0.04). Age, year of diagnosis, relationship status and extent of disease were not significantly associated with particular attitudes.

Conclusion: Most respondents judged presentation of best case, worst case and most likely scenarios preferable, more helpful and more reassuring than presentation of just the median survival time when oncologists explain prognosis to people with advanced cancer.

P5-15-03

Development of a Patient Decision Aid for Women 70 Years Old and Older with Stage I, Hormonally Sensitive, Breast Cancer Considering Adjuvant Treatment Post-Lumpectomy.

Szumacher E, Wong J, D’Alimont E, Angus J, Passat L, Metcalfe K, Thelan T, Llewellyn-Thomas H. Odette Cancer Centre, Sunnybrook Health Sciences Centre, Toronto, ON, Canada; University of Toronto, Toronto, ON, Canada; Education Independent Consultant, Toronto, ON, Canada; Juravinski Cancer Centre, Hamilton, ON, Canada; Women’s College Research Institute, Toronto, ON, Canada; Dartmouth Medical School, Lebanon, NH

Background: Decision Aids (DA) are developed with the intent to support people in making specific and deliberate choices by improving information transfer about different outcomes. Previous research has shown that DAs can increase patient knowledge regarding treatment options, reduce decisional conflict, and increase patient satisfaction with the decision-making process. However, no DAs have been developed to help older breast cancer patients decide whether or not to undergo adjuvant RT. We developed and tested a DA for older women with stage I, ER/PR positive breast cancer considering adjuvant treatment post-lumpectomy and we examined its impact on treatment decision-making process.

Methods and Materials: A DA was developed and evaluated in three steps following the Ottawa Decision Aid Framework: 1) Needs assessment (N=16); 2) Pilot I, to examine the DA’s acceptability (N=12); and 3) Pilot II, a pre-test post-test (N=38) with older women with ER/PR responsive breast cancer post-lumpectomy who were receiving adjuvant RT. Measures included questionnaires to assess patient’s satisfaction with the DA, patients’ self-reported decisional conflict (DC), level of distress, treatment-related knowledge, and choice predisposition.

Results: The DA is a booklet that details each adjuvant treatment option’s benefits, risks and side-effects tailored to their clinical profile; includes a value clarification exercise; and steps to guide them towards their own treatment decision. All women felt the DA was helpful and informative. Compared with baseline scores, patients had a statistically significant (p < .05) reduction in DC (adjusted mean difference [AMD], -7.18; 95% confidence interval [CI], -13.50 to 12.59); increased clarity of the treatment benefits and risks.
Discussion: This study provides evidence that this DA may be a helpful educational tool for this group of women. The quality of care for older breast cancer patients may be enhanced by using a tailored DA to help the patient be informed of their treatment options and to prepare for decision-making.

P5-15-04
Withdrawn by Author

P5-15-05

Boughey JC, Hoskin TL, Williams CI, Hartmann LC, Allers TM, Degnim AC, Frost M. Mayo Clinic, Rochester, MN

Background: Women with hereditary breast cancer risk, who develop breast cancer, face complex surgical decisions related to their breast cancer and risk to their contralateral breast. The aim of this study was to prospectively evaluate the factors influencing decision making in these women and degree of decision conflict before and after consultation with breast specialized physicians (BSP).

Methods: Women with hereditary breast cancer risk and recently diagnosed with unilateral breast cancer were enrolled in a prospective decision making questionnaire study. Questionnaires were completed prior to their initial breast clinic visit and after consultation with a breast clinic physician, nurse educator and a breast surgeon.

Results: 68 women completed both questionnaires. Mean age was 52 (range 27-84). Prior to meeting with BSP, 10 (15%) women reported having made a decision for the cancer side and 18 (26%) reported being close to deciding. Of the 20 who expressed a surgical preference prior to consultation, 5 (25%) changed their decision after meeting with the BSP [1 to mastectomy (MTX), 4 to breast conservation (BC)]. After consultation with BSP, 65 (96%) reported having made a decision (BC in 29 (45%), MTX in 35 (54%), unspecified in one).

The most commonly cited reasons influencing surgical choice were doctor’s advice (79%), family history of breast cancer (74%) and worrisome findings on biopsy (50%).

Prior to meeting with BSP, 9 (13%) women reported having made a decision regarding screening and/or risk-reducing options for the contralateral breast and another 11 (16%) reported being close to a decision. The choices in these 20 women were screening only in 10 (50%), contralateral prophylactic MTX (CPM) in 3 (15%), CPM and oophorectomy in 2 (10%), other combinations of options in 4, and unspecified in 1. After meeting with the BSP, 49 (72%) reported having made a decision regarding the contralateral breast; this decision was for screening only in 21 (43%), CPM only in 9 (18%), screening plus tamoxifen in 7 (14%), CPM and oophorectomy in 2 (4%), other combinations of options in 8, and unspecified in 2.

The most commonly cited reasons for choices on the contralateral side were: family history (81%), doctor’s advice (78%), and worrisome findings on biopsy (43%); only 25% cited psychological or emotional reasons.

For both the cancer and contralateral sides, decisional conflict was significantly lower after meeting with BSP than prior to meeting with BSP.

<table>
<thead>
<tr>
<th>Decision-conflict scale total scores (possible range 0-100; higher scores indicate more decisional conflict) summarized for each side and compared between timepoints</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
</tr>
<tr>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>Cancer side</td>
</tr>
<tr>
<td>Contralateral side</td>
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</table>

Conclusion: The BSP consult and family history have the greatest influence on decision making among women with hereditary risk diagnosed with breast cancer. After consultation most women felt they had made decisions for both the cancer and contralateral sides and reported significantly lower decision conflict compared to prior to the consultation.

P5-16-01
Are We Going To Be Able To Train Future Surgeons How To Perform Axillary Lymph Node Dissection?

Betambeau N, Chan C. Cheltenham General Hospital, Cheltenham, Gloucestershire, United Kingdom

Background: Axillary lymph node dissection (ALND) has been performed regularly by surgeons for several decades. The sentinel lymph node era has led to a reduction in ALND procedures as node negative women are now spared further surgery and morbidity. Following the publication of the ACOSOG Z0011 trial, it is conceivable that completion ALND (cALND) may be rendered unnecessary for many women who have breast conserving surgery and have 1 to 2 positive sentinel lymph node disease. If there is a substantial drop in ALND procedures, this may impact on our ability to train surgeons in the future.

Material and Methods: We retrospectively reviewed the breast cancer practice for 2 consultant surgeons in a busy UK centre. In this centre all patients with a positive diagnosis of invasive breast cancer had pre-operative axillary ultrasound scanning (USS) and needle biopsy (fine needle aspiration or core biopsy) if enlarged or suspicious axillary lymph nodes were seen. Hence sentinel lymph node biopsy (SLNB) was only performed on patients with both clinically and radiologically negative axillary lymph nodes.

Results: In 2009 and 2010 the 2 consultants treated a total of 255 patients with invasive breast cancer. Thirty one patients (12.2%) had positive axillary lymph disease identified by USS and axillary needle biopsy; these women were treated with ALND. The remaining 224 patients had clinically and radiologically negative axillary lymph nodes and proceeded to SLNB. Of these, 58 patients had at least one positive sentinel lymph node (25.9%) and proceeded to cALND; 39 women had breast conserving surgery and 19 women had mastectomy. If our centre were to adopt a policy of observation only in those women having breast conserving surgery and whom had no more than 2 positive sentinel lymph nodes (as per ACOSOG Z0011 trial), then 33 fewer ALND would have been performed. This would leave a new total of 56 ALND procedures being performed over a 2 year period in women with pre-operatively identified positive axillary lymph nodes, those undergoing mastectomy and those women with 3 or greater positive sentinel lymph nodes on SLNB. On our service we have 2 residents which would result in a maximum number of 14 ALND procedures per year that each resident may be trained to perform. It is likely that the actual number will be less due to commitments away from the elective operating room, such as emergency duties and annual leave etc. Is this an adequate number of ALND procedures for a resident to gain competence in the technique?

Discussion: The training of surgical residents is competence based and if our centre is representative of most centres treating breast cancers in the UK, will the teaching of ALND procedures be limited to specialist, ultra-high volume centres if the ACOSOG Z0011 trial findings are to be adopted?
P5-16-02
The Impact of Educational Materials on Compliance and Persistence with Adjuvant Aromatase Inhibitors: 2 Year Follow-Up and Final Results from the CARIATIDE Study.

Neven P, Markopoulos C, Tanner MME, Marty ME, Kreienberg R, Atkins L, Franquet AA, Serin D, Culcelik MA, Deschamp V. University Hospitals Leuven, Leuven, Belgium; Medical School University of Athens, Athens, Greece; Tampere University Hospital, Tampere, Finland; Saint Louis University Hospital, Paris, France; University of Ulm, Ulm, Bade-Wurttemberg, Germany; University College London, London, United Kingdom; IDDI (International Drug Development Institute), Louvain-la-Neuve (Ottignies), Belgium; Institut St. Catherine, Avignon, France; Ankara Oncology Hospital, Ankara, Turkey; AstraZeneca, Brussels, Belgium

Rationale
Understanding and effectively addressing the factors that affect patient compliance with adjuvant aromatase inhibitors (AI) is required in order for patients to obtain maximum benefit from treatment. The CARIATIDE study sought to determine whether the provision of educational materials (EM) could improve compliance and persistence with adjuvant AI. At 1-y follow-up (FU), there was no improvement in overall compliance with AI therapy, compliance with initial AI or persistence rates when EM were provided. Final results from the 2-y FU are presented here.

Methods
This 2-y, global observational study (NCT00681122) randomized 2758 patients, across 18 countries, to Group A: Standard Therapy or Group B: Standard Therapy + EM. The EM were developed in collaboration with patient advocates, and consisted of a range of information on breast cancer-related topics. Compliance rate with adjuvant AI medication was the primary endpoint. Secondary endpoints included persistence rate after 1 and 2y, and reasons for, and time to, discontinuation of AI therapy. Compliance rate was defined as the proportion of patients being ‘compliant’ with the adjuvant AI medication; switching from AI therapy to tamoxifen would result in a non-compliance score at time of switching. For compliance with initial adjuvant AI medication, switching to another AI or hormone therapy would result in a non-compliance score. A patient was considered a ‘persistent’ user if they did not switch AI medication, AI medication was uninterrupted and there was no discontinuation of the AI medication during the second year. Patients’ compliance and behavior were evaluated using compliance questionnaires, EM feedback and validated questionnaires (EORTC IN-PATSAT32, GHQ-12, FACT-ES).

Results
Of the 2758 patients randomized at study initiation, 2242 were available for analysis at 2-y FU. The results confirmed those obtained at 1-y FU. No statistically significant difference in compliance with AI therapy was observed between Group A and Group B (82% and 82%, respectively, p=0.9926). Compliance with initial AI was 81% in Group A and 80% in Group B (p=0.5541), with persistence rates of 90% and 88%, respectively (p=0.2425). Of the proportion of patients who had compliance data for both years (Group A n=1118; Group B n=1111) 72% were compliant for the whole 2-y FU period. Across the full 2-y FU, AI treatment discontinuation rates of 8% and 9% were observed in Group A and B, respectively, with discontinuation most frequently attributed to AI-related side effects. Analysis showed that no specific baseline demographic characteristics were associated with compliance behavior. Compliance rates differed widely between countries.

Conclusions
At 2-y FU, EM were not found to improve overall patient compliance, compliance with initial AI, or persistence with therapy. In total, 72% of patients were compliant across the full 2-y FU. AI-related side effects remained the most frequent cause of AI treatment discontinuation across the full FU period. The 2-y CARIATIDE data confirm the 1-y findings.

P5-16-03
The Breast Cancer Novela, Se Valiente...Son Tus Senos, an Innovative Tool To Educate Latina Women about Breast Cancer. Fehus-Sampayo I, Mota R, Yaker A. SHARE: Self-Help for Women with Breast or Ovarian Cancer, New York, NY

Background:
The Hispanic population is the fastest growing population and largest minority group in the United States. Breast cancer remains the leading cause of cancer death among latina women in part because they are more frequently diagnosed at a later stage than non-Hispanic whites. Critical information about early detection, diagnosis and treatment options is often inaccessible due to cultural and language barriers. There is a need to reach the Hispanic community in new and compelling ways. The purpose of this initiative was to reach diverse Spanish speaking communities, particularly in New York City, with information about breast health and breast cancer that was culturally-sensitive, language appropriate, scientifically and medically accurate and, most importantly, relevant to the lives of the people in the community.

Methods:
The Hispanic population is the fastest growing population and largest minority group in the United States. Breast cancer remains the leading cause of cancer death among latina women in part because they are more frequently diagnosed at a later stage than non-Hispanic whites. Critical information about early detection, diagnosis and treatment options is often inaccessible due to cultural and language barriers. There is a need to reach the Hispanic community in new and compelling ways. The purpose of this initiative was to reach diverse Spanish speaking communities, particularly in New York City, with information about breast health and breast cancer that was culturally-sensitive, language appropriate, scientifically and medically accurate and, most importantly, relevant to the lives of the people in the community.

Methods:
Latina breast cancer survivors and non-survivors from diverse Hispanic communities were identified and three 8 person focus groups, two for non-survivors and one for survivors, were organized to explore breast cancer-related issues relevant to these communities. The women’s personal stories and common issues and experiences as well as medical information reviewed by a medical advisory group, formed the basis for the development of a breast cancer novela. The novela is a popular Spanish-language comic book style publication widely distributed in the Hispanic communities. The breast cancer novela used the real life stories of the women to address barriers to accessing health care and empower women in Hispanic communities. A distribution plan was developed and a curriculum created for in-person educational seminars at community organizations. Surveys prior to and following the educational intervention were conducted.

Results:
We distributed over 25,000 novelas in Spanish to 478 sites within New York City and to 89 other sites throughout the U.S. We printed and distributed 2500 copies of an English version of the novela at the request of health providers. Attendees at a launch event helped identify distribution sites and educational seminar venues. Peer facilitators conducted these seminars, with the novela as the primary communication tool. The content of the workshops included information about breast health, risk, early detection, myths and realities about a diagnosis, managing relationships, survivorship and resources. To date, 17 seminars have been conducted and attended by more than 330 attendees. Results of the surveys as well as detailed information describing the distribution sites of the novela and feedback we received about its impact, will be presented at SABCS

Discussion:
Se Valiente...Son Tus Senos, is an innovative educational tool that conveys information about breast health, breast cancer detection, diagnosis, treatment and survivorship that is personal, relevant, compelling and accessible. The breast cancer novela serves as a vehicle to change information levels and increase women’s knowledge and to provide health providers with insights that enable them to treat members of Spanish-speaking communities in a culturally competent manner.
P5-17-01
Evaluation of Psychosocial Distress in Main Care-Givers of Patients with Metastatic Breast Cancer Who Receive Treatment in a Community Based Oncology Group Practice.
Weide R, Feiten S, Friesenhahn V, Heymanns J, Kleboth K, Mergenthaler U, Thomalla J, van Roye C, Köppler H. Hematology/Oncology Group Practice, Koblenz, Germany; Institute for Health Care Research in Oncology, Koblenz, Germany

Introduction: It is well-known that people who care for patients with a metastatic carcinoma are exposed to an above-average level of psychosocial distress. No data are available concerning the distress of main care-givers of female patients with metastatic breast cancer, who are treated in a community based oncology group practice.

Methods: Standardized cross-sectional survey of main care-givers and patients with metastatic breast cancer who were treated in a community based oncology group practice in Germany between 04/2010-03/2011. Psychosocial distress of the patients and their main care-givers were evaluated using the German versions of the Distress Thermometer (DT) and the Problem List (PL). In addition anxiety and depression of the main care-givers were assessed using the Hospital Anxiety and Depression Scale (HADS-D).

Results: 83 female patients with a median age of 65 (41-93) were interviewed. 6% did not have a main care-giver, 7% indicated that they needed no support, 48% reported one main care-giver and 39% several. Partners (60%), children (47%), siblings (11%) and friends (10%) were the most important care-givers. 47% of patients preferred visiting the practice in companion with their care-givers. The patients' median score on the DT was 5 (0-10), with 34% scoring above cut-off (>5) for psychosocial distress.

52 main care-givers (61% male, 39% female) with a median age of 57.5 (41-86) were interviewed. The relationships to the patients were as follows: partners 62%, children 27%, mothers, siblings and friends each with 4%. The main care-givers themselves were supported by partners (54%), children (21%) friends (17%) and siblings (8%), 23% did not receive any support. The median score on the DT was 5 (0-10), with 44% scoring above cut-off (>5) for psychosocial distress. According to the HADS-D 37% (cut-off ≥8) of the care-givers reported anxiety, with a mean score of 6.6 (0-14). 15% could be regarded as depressed (cut-off ≥8), with a mean score of 4.1 (0-15).

Conclusions: The main care-givers are distressed even more than the patients themselves. 37% of care-givers reported anxiety; depression can be observed too, but less frequently in 15%. Both issues should be addressed by healthcare professionals.

P5-17-02
Associations between Breast Cancer Patients’ Satisfaction with Nursing Staff and Hospital Characteristics, Results of a German Multicenter Study.
Wuerstlein R, Kowalski C, Diener S, Krebs S, Pfaff H, Harbeck N. University Hospital Cologne, CIO, Köln, Germany

Background: Only few studies have investigated the association between breast center characteristics such as teaching status, number of patients, whether clinical trials are conducted, or whether breast care nurses are employed and patients’ perceptions of care.

Objective: The aim of the study was to determine whether or not the satisfaction of newly-diagnosed breast cancer patients with nursing staff correlates with hospital characteristics after adjusting for relevant patient characteristics. The study is part of a national process-identifying project focusing on breast cancer of the MAGS NRW (Ministry of work, health and social affairs, North Rhine Westfalia, Germany).

Methods: Multilevel regression analysis was applied combining data from newly-diagnosed breast cancer patients (n= 3733) on patient characteristics and satisfaction with nursing staff and data on characteristics of the hospitals (n= 93 breast centers in Germany) in which patients were treated. Patients’ perspectives were measured for six month using the Cologne Patient Questionnaire for Breast Cancer (CPQ-BC), the other informations and hospital characteristics were collected by medical personnel.

Results: Full data of 2945 patients from 81 hospitals were analysed in the multilevel logistic regression model. The odds for patients being satisfied with nursing staff were significantly higher in hospitals which employed breast care nurses at the time of the survey. At the patient level, patients were significantly more likely to be satisfied with nursing staff if they were native speakers, and with higher self-rated health. Cross-level interaction analysis suggested that higher patient satisfaction resulting from employing breast care nurses was largely limited to native speaking patients. Other hospital characteristics were not significantly associated with the perception of nursing staff (teaching hospital, required number of surgeries, clinical trial participation).

Conclusions: The results demonstrate that patient satisfaction with nursing staff is higher if breast care nurses are employed in the treating hospital. Further work will focus on the description of the breast care nurses work as it is interpreted individually in every breast center. However, only the satisfaction of native speakers was significantly higher when breast care nurses were employed.

Implications for Practice: Findings suggest that hospitals should invest in employing specialist nurses. Special attention should be paid to the care of non-native speaking patients.

P5-17-03
The Association between Breast Cancer Related Lymphedema’s Risk Factors and Likelihood of Edema Progression.
Skolny MN, Miller CL, O’Toole J, Sadek B, Ancukiewicz M, Taghian AG. Massachusetts General Hospital, Boston, MA

Purpose/Objective(s): Breast cancer related lymphedema (BCRL) is one of the most feared long term side effects of treatment. Although emergence of the sentinel lymph node biopsy (SLNB) has decreased the rate of BCRL, it continues to be an issue for this population. The goal of this study is to evaluate the prognostic risk factors for BCRL development and progression based on longitudinal prospective limb volume (LV) measurements and associated surgical, radiation and medical oncology factors.

Materials/Methods: Since 2005 newly diagnosed breast cancer (BC) patients were prospectively screen via perometry (Pero-System). For this analysis patients were required to have undergone at least four measurements and have at least 24 months of follow up to allow for adequate time for LE development. Bilateral pre-operative volume measurements of the upper extremity were compared to post-operative measurements and at 4 to 6 month intervals to assess for limb volume changes. At each time point (tx), relative volume change (RVC) of the treated arm was calculated using volume ratios of treated (T) to non-treated (N) side compared to the pre-operative baseline (t1) (RVC=(Tx/Ntx)/(Tt1/Nt1)-1) as described in Ancukiewicz et al 2010. Our institution utilizes >5% RVC as an indicator of lymphedema. For this analysis, mild lymphedema was classified as a RVC of 5-9.9% and moderate lymphedema was classified as a RVC ≥10%. A univariate analysis was performed to identify risk factors and likelihood to LE progression.

Results: In a cohort of 415 women, 14.4 % (60) and 6.7% (28) developed mild and moderate BCRL, respectively. Twenty percent
(14) and 75 % (21) of patients in the mild and moderate cohorts, respectively, had a persistent edema that was documented at least 2 consecutive data points. Eighty percent (56) and 25% (7) of patients that had a documented incidence of an elevated RVC had returned below an RVC<5% and their subsequent visits. Based on Kendall’s Tau P-value of <0.0001 persistent edemas were associated with axillary lymph node dissection (ALND), number of lymph nodes removed, axillary fields and dose of radiation.

Conclusion: Breast cancer related lymphedema is a feared long term side effects and negatively impacts the physical and psychological aspects of an individual’s life. A condition that is considered manageable but not curable, it is essential providers identify high risk individuals. Establishing a a lymphedema screening program may identify early lymphedema which could be potentially be treated successfully.

P5-17-04
Association between Breast Cancer and Osteoporosis among Women 85 Years or Older.

Okanami Y, Homma N, Arai T, Sawabe M, Maeda I, Takagi M, Younes M, Takubo K. St. Marianna University School of Medicine, Kawasaki, Kanagawa, Japan; Tokyo Metropolitan Institute of Gerontology; Baylor College of Medicine

Background: The pathogenesis of breast cancer in very elderly women is of interest, because estrogen levels are likely to be extremely low during the development of the disease. Estrogens have opposing effects on the pathogenesis of breast cancer and osteoporosis. In an effort to understand the significance of estrogens in the pathogenesis of breast cancer in this group, we examined the association between breast cancer and osteoporosis in women at least 85 years old, taking body mass index (BMI) and hormone receptors status into consideration.

Methods: Clinical records of consecutive elderly women (≥ 85 y/o) who underwent breast cancer surgery at Tokyo Metropolitan Geriatric Hospital (BC) and control women of the same age group (Cont) were reviewed; osteoporosis and BMI status in both groups were recorded and compared. The status of estrogen receptor (ER) and progesterone receptor (PR) of breast cancers was examined immunohistochemically and compared between BC with and without osteoporosis (BC-OP and BC-nonOP, respectively).

Results: Frequency of osteoporosis was significantly lower among BC than Cont. There was no difference in BMI measured after 85 y/o between BC and Cont; however, percentage of women, whose BMI measured when they were in their sixties or seventies was ≥ 20% higher than BMI measured when they were 85 y/o or older, was significantly higher among BC than Cont (31% and 2%, respectively. P = 0.0001). ER positivity of breast cancer did not differ between BC-OP and BC-nonOP, whereas PR positivity was significantly higher among BC-nonOP than BC-OP (P = 0.0359).

Discussion: The inverse relationship between the incidence of breast cancer and osteoporosis suggests an important role for estrogens in the pathogenesis of breast cancer in the very elderly women. Our finding that one third of BC had ≥ 20% higher BMI when they were in their sixties or seventies suggests they may also have elevated serum estrogens during these earlier years, because increased BMI has been reported to be associated with increased serum estrogens in postmenopausal women. Expression of PR is regulated by estrogen-ER signaling, and PR positivity is considered to reflect the effectiveness of estrogen-ER pathway. Significant difference of PR positivity between BC-nonOP and BC-OP suggests the more importance of estrogens in BC-nonOP than BC-OP.

Conclusion: Estrogens seem to play an important role in the pathogenesis of breast cancers in very elderly women, especially without osteoporosis. With increasing aging population in developed countries, those findings may have implications for breast cancer prevention in this age group.

P5-17-05
24 Months Follow-Up Results from PACT (Patient's Anastrozole Compliance to Therapy Programme), a Non-Interventional Study Evaluating the Influence of a Standardized Information Service on Compliance in Postmenopausal Women with Early Breast Cancer.

Lueck H-J, Hadji P, Harbeck N, Jackisch C, Blettner M, Zaun S, Windemuth-Kieselbach C, Beck T, Köhler U, Schmitt D, Krüenberg R. Gyn-Oncological Practice, Hannover, Germany; Phillips-University, Marburg, Germany; Breast Center, University of Cologne, Cologne, Germany; City Womens Hospital Offenbach, Offenbach, Germany; Institute of Medical Biostatistics, University, Mainz, Germany; AstraZeneca Germany, Wedel, Germany; Alcedis GmbH, Germany; Hospital Rosenheim, Rosenheim, Germany; Hospital St. Georg, Leipzig, Germany; Consulting Doctor-Patient Communication, Radolfzell, Germany; University Womens’ Hospital, Ulm, Germany

Introduction: According to recent retrospective studies, compliance to adjuvant endocrine therapy for early breast cancer (EBC) may drop to below 70% after one year and to as low as 50% by year 4. PACT aimed to increase treatment adherence in postmenopausal women taking an adjuvant aromatase inhibitor via a standardized information service (educational arm). Yet, after 12 months, there was no difference in compliance between the standard and educational arm (reported at this meeting 2010). Methods: PACT is a prospective, randomised, two-arm parallel-group study in Germany, sponsored by AstraZeneca (NCT00555867). Postmenopausal women on anastrozole for hormone-receptor positive (HR+) EBC were randomized to routine clinical care alone or to receive additional standardized information service (educational arm) at nine times over the first 12 months of adjuvant therapy. Primary endpoints were compliance and persistence rates in the educational versus routine arm after 12 months. Secondary endpoints included longer follow-up, reasons for non-compliance, influence of baseline characteristics, and clinical outcome parameters (DFS, OS). Compliance was evaluated via patient questionnaires, prescription data and physician recall. Per protocol compliance was analysed only for patients with full documentation both by patients and physicians. Persistence was defined as the duration of time from initiation to discontinuation of therapy (Cramer et al 2007) and measured by prescription data.

Results: PACT enrolled 4,923 female patients at 109 breast centres and 1,361 registered specialist practices from all regions in Germany. 4,397 patients were evaluable for baseline characteristics. 2,707 patients were evaluable for the primary endpoint (full documentation on tablet intake both by patients and physicians). No difference in compliance could be shown between the standard (88.2%) and the educational arm (88.3%) at 12 months (p=0.92, Fisher’s exact test). Compliance rates were 40.3% for the standard arm and 43.0% for the educational arm, respectively (p=0.17, Fisher’s exact test). At 24 months, data from 1539 patients was available for analysis per protocol. Compliance rates were 88.7% (educational arm) and 87% (standard arm; p=0.29). Persistence again much lower at 41.1% and 42.1% (p=0.68). Variables influencing compliance were regular attendance to follow-up visits, participation in a cancer rehabilitation program, number of co-morbidities and current employment status. Persistence was influenced by factors such as tumour stage, joint pain...
and cancer rehab program participation. **Conclusion:** The addition of standardized information materials to standard clinical care did not lead to a significant increase in compliance or persistence rates at 12 or 24 months. With 4,923 women included, the PACT study represents the largest prospective study to evaluate the influence of educational material as well as baseline demographic and histopathological characteristics on the compliance and persistence to adjuvant endocrine therapy in postmenopausal patients with HR+ EBC.

**P5-17-06**

Pilot Study of a Questionnaire To Assess Impact of a Breast Cancer Diagnosis in Young Women on Their Relationship with Their Mothers.

Ali A, Fergus K, Wright F, Pritchard K, Kiss A, Warner E. Sunnybrook Health Sciences Centre, Toronto, ON, Canada

**Background:** Breast cancer in young women (≤40) is associated with greater morbidity, both physical and psychological, and mortality than in older women. For women in their 20s and 30s we would expect the mother-daughter relationship to be uniquely impacted by breast cancer. However, no study has apparently looked at the effects of a breast cancer diagnosis in this situation. We completed a pilot study to assess the clarity, content and sensitivity of a questionnaire that will be used in a comprehensive assessment of this relationship in young women with breast cancer. **Methods:** A questionnaire with a mixture of Likert and open-ended items was developed after a literature review, obtaining input from an interdisciplinary panel of experts in psychology, medical oncology, research methodology, and breast cancer, and informal interviews with 3 young breast cancer survivors. Topics covered include demographics, nature of the mother-daughter relationship pre and post diagnosis and sources of support to daughters and mothers. Ten breast cancer survivors (≤ age 40 at diagnosis and > 36 months after their diagnosis) were asked to assess the questionnaire. **Results:** Ten patients, median age 37 years (range 31-52), participated in the pilot study. The average time to complete the questionnaire was about 30 minutes. No questions were reported to be upsetting. All but 1 patient indicated that the questionnaire was clear. The questionnaire was modified based on patient feedback. The number of items was reduced from 51 to 38. Items concerning, for example, nature of the mother-daughter relationship during childhood or teenage years, and openness to discussing personal gynecological matters prior to breast cancer diagnosis were ultimately excluded. A question inquiring about the most difficult issues the daughter faced after diagnosis was added. Some questions were changed from open ended format to relevant tick box options after reaching response item saturation during the pilot phase. Of the 10 patients, 8 felt close to their mothers in the year prior to their diagnosis. Seven patients reported turning to their mothers for support when they first learned of the diagnosis, and 4 stated their mother was the first person they turned to. All 10 felt their mothers were emotionally and practically supportive. Five indicated that their relationship got closer post-diagnosis. One patient reported that her mother was not coping, and 3 indicated that their mothers did not have adequate support. Many patients felt that health care providers could help mothers by being empathetic, providing information, and linking mothers with supports. **Discussion:** The mother-daughter relationship is an important source of support for young breast cancer survivors. Our questionnaire will be used in 100 women ≤ age 40 and within 3 years of diagnosis to further study the impact of breast cancer in a young woman on the mother-daughter relationship and to determine whether any intervention targeted to the mothers (eg. information or support groups) might be helpful.

**P5-17-07**

HER2+ Metastatic Breast Cancer Patient Experiences on Treatment in the Biologic Era: Findings from a Community Web-Based Survey.

Mayer M, Doan JF, Lang K, Hurvitz SA, Lalla D, Woodward RM, Bramer MG, Menzin J, Tripathy D. AdvancedBC.org; Genentech, Inc., South San Francisco, CA; Boston Health Economics, Waltham, MA; UCLA Jonsson Comprehensive Cancer Center, Los Angeles, CA; USC/Norris Comprehensive Cancer Center, Los Angeles, CA

**Background:** HER2-directed biologic therapy is part of recommended treatment for women with HER2+ metastatic breast cancer (MBC). However, little is known regarding the quality of life (QoL) experience and symptom burden in these women outside of clinical trials. **Methods:** This one-time, web-based survey was conducted with 6 independent U.S. breast cancer support groups. Respondents were required to be female, aged 18+ with HER2+ MBC, and to have received active treatment in the past month outside of a clinical trial. Data were collected on demographic, clinical, employment, QoL (Rotterdam Symptom Checklist and EQ-5D), and other measures. Survey responses were stratified by active treatment (biologic alone, biologic in combination with other therapy, no biologic) and number of months since diagnosis of MBC. **Results:** Of 337 possible respondents, 185 women with HER2+ MBC completed the survey. The majority were aged 45-59 years (53.5%), white (94.6%), living with a spouse or partner (74.6%), and had at least some college education (93.5%). Most (64.9%) reported bone as a site of metastasis, followed by liver (36.2%), lung (31.9%), and brain (21.6%). During the prior month, 60 respondents (32.4%) had received biologic therapy only (trastuzumab, lapatinib and/or bevacizumab), 93 (50.3%) received biologic with another treatment, 21 (11.4%) received no biologic and 11 (5.9%) did not report a specific active treatment. Demographic and clinical characteristics were similar across therapy groups, with some geographic variation. Overall, the tumor receptor status was ER+/PR+ for 43.2% of respondents. Mean months since MBC diagnosis ranged from 46 among those taking a biologic with another treatment to 60 for those taking biologics only. Average Rotterdam subscale scores indicate that psychological symptoms caused the greatest impairment (mean= 0.8, P<.05), followed by physical symptoms (74.1) and activity limitations (87.3). There were no clinically meaningful (≥8 point) differences across biologic groups, with the greatest difference between the biologic with other therapy (73.5) and biologic-only (80.8) groups for “overall evaluation of life.” The average EQ-5D utility index score was 0.8 (1.0 is perfect health), with minimal variation across therapy groups. Most respondents reported no problems with mobility (62-77%) or self-care (≥90% across groups). However, pain/discomfort and anxiety/depression were problematic for more than 50% of all respondents, and were most commonly reported among the biologic plus other treatment group (61.3% and 51.6% respectively, P<.05 for differences in usual activity problems). Rotterdam and EQ-5D dimension scores indicated less impairment with more time since MBC diagnosis, particularly for EQ-5D mobility, self-care, usual activities, and pain/discomfort. Pain and mobility scores for those diagnosed 72+ months ago were significantly better than for those diagnosed 0-17 months ago (both P<.05). **Conclusions:** This community survey of women with HER2+ MBC provides valuable insight into treatment, quality of life and symptom burden. Pain and psychological issues continue to be challenges for this population.
P5-17-08
Hurvitz SA, Mathias SD, Doan JF, Crosby RD. UCLA School of Medicine/Translational Oncology Research International, Los Angeles, CA; Health Outcomes Solutions, Winter Park, FL; Genentech, Inc., South San Francisco, CA

Background: While PFS indicates the period of time a patient’s disease does not progress, there is debate about the relative value of longer PFS in the absence of a meaningful difference in OS. However, there are no known published reports about the value of PFS from the patient’s perspective.

Material and Methods: This was a 2-part project consisting of instrument development (via focus groups) and cross-sectional administration to 2 groups of women, those who had vs. those who had not experienced disease progression. Based on input from 19 women with MBC in the US, we developed an MBC-specific questionnaire (MBC-P). The questionnaire focuses on unique aspects relevant to MBC patients including concerns about the future, social isolation, fear, activity limitations, mental health, social functioning, and activity limitations. The MBC-P also presented descriptions of two hypothetical treatments: Treatment X, associated with 12 mos of PFS and Treatment Y with 16 mos of PFS, which had the same side effect profile. Respondents were asked to state which treatment was associated with better overall quality of life (O-QOL), physical functioning (PF), emotional well being (EWB), and which treatment was preferred. We compared mean scores at the item level for respondents based on progression status. One final aspect of the questionnaire asked respondents to rate (on a 0-100 scale) O-QOL, PF, EWB at the time of initial breast cancer (BC) diagnosis, MBC diagnosis, and MBC progression (if applicable). Ethics approval was obtained.

Results: 282 women completed the survey [mean age: 50 yrs (range: 21-80)]; 56% had experienced progressive disease. Women whose disease had progressed were more likely (p < 0.05) to report putting their life on hold, worrying about there not being a treatment that will work, feeling less confident about their treatment, and experiencing greater limitations in recreational or physical activities and accomplishing things/tasks. For the hypothetical descriptions, no differences emerged based on progression status. Despite being told that side effects and OS were the same for both scenarios, significantly more women responded that treatment Y (vs. treatment X) would result in better QOL, physical functioning, and emotional well being. Additionally, 88% of respondents preferred treatment Y over treatment X. Ratings for different stages of disease on O-QOL, PF and EWB were lowest the first time a woman was told her MBC had progressed and highest when told she had responded to treatment (O-QOL: 58 vs. 77; PF: 61 vs. 71; EWB: 51 vs. 77).

Discussion: The MBC-P questionnaire with input from women with MBC and is a useful tool to evaluate the value of PFS from the patient’s perspective. Women with MBC whose cancer had progressed were more limited in areas pertaining to treatment and activity limitations compared to women who had not experienced progression. Surprisingly, even when told that side effects and OS were similar, women rated O-QOL, PF, and EWB as being better for a hypothetical drug that provided 4 additional months of PFS. Results should be confirmed with a larger, more diverse sample of MBC patients and eventually in a prospective study.

P5-18-01
No Effect of Adjuvant Chemotherapy in Postmenopausal Patients with Invasive Lobular (Mixed) Breast Cancer.
Troll W, Voogd A, Vreugdenhil G, Van der Heiden-van der Loo M, Siesling S, Roumen R. Maxima Medical Centre, Veldhoven, Netherlands; Maastricht University, Maastricht, Netherlands; Comprehensive Cancer Centre, Utrecht, Netherlands; Comprehensive Cancer Centre, Enschede, Netherlands

Background: Although the literature is clear concerning the lack of response of invasive lobular breast cancer to neoadjuvant chemotherapy, no studies exist on the effectiveness of the adjuvant use of chemotherapy in these patients, as compared to those with ductal and mixed type lobular cancer.

Methods: All women with primary non-metastatic invasive ductal or (mixed type) lobular breast cancer, aged 50 to 69 years, diagnosed in the period 1995 to 2008 and treated with surgery, were selected from the Netherlands Cancer Registry and followed until January 1, 2010. Patients were divided in two groups: those who received adjuvant hormonal therapy only versus those receiving hormonal therapy in combination with adjuvant chemotherapy. Cox proportional hazards analyses were carried out to determine the impact of chemotherapy in addition to hormonal treatment, for each histological entity separately.

Results: In total 19,609 patients had ductal cancer, 3,685 had pure lobular cancer and 1,391 had mixed type lobular cancer. The patient groups were comparable with respect to the use of adjuvant systemic treatment. The 10-year survival of patients with ductal cancer treated with hormonal therapy alone was 69%, compared to 74% for those treated with hormonal therapy and chemotherapy (P<0.0001). For patients with lobular cancer, the 10-year survival rate was 68% after hormonal treatment alone and 66% after hormonal therapy with chemotherapy (P=0.45). The hazard ratio for death among the patients with ductal cancer receiving chemotherapy in addition to hormonal treatment was 0.70 (95% CI, 0.64-0.76, p<0.0001), as compared to those receiving hormonal treatment alone. In patients with pure or mixed type lobular cancer, however, the hazard ratio’s were 1.00 (95% CI, 0.82-1.21, p=0.97) and 0.98 (95% CI, 0.70-1.33, p=0.83), respectively. A statistically significant interaction was observed between the use of adjuvant chemotherapy and histological tumor type.

Conclusions: Adjuvant chemotherapy confers no additional beneficial effects in postmenopausal patients with pure or mixed type lobular breast cancer receiving hormonal therapy.

P5-18-02
A Population Level Assessment of Emergency Room Visits and Hospitalizations for Women Undergoing Adjuvant Chemotherapy for Early Breast Cancer.
Enright KA, Trudeau M, Yun L, Grunfeld E, Krzyzanowska M. Peel Regional Cancer Centre, Credit Valley Hospital, Mississauga, ON, Canada; Sunnybrook Odette Cancer Centre, Toronto, ON, Canada; Institute for Clinical Evaluative Science, Toronto, ON, Canada; University of Toronto, Toronto, ON, Canada; Cancer Care Ontario, Toronto, ON, Canada; Princess Margaret Hospital, Toronto, ON, Canada

Background: Adjuvant chemotherapy is considered the standard of care for women with lymph node positive and high risk lymph node negative breast cancer. While the acute toxicities of chemotherapy are well documented in clinical trials, the frequency of serious treatment related toxicities of adjuvant chemotherapy in the general population is not well described. We undertook a population based assessment of the frequency of serious treatment related toxicity in women
undergoing adjuvant chemotherapy for early breast cancer (EBC).

Methods: All incident EBC patients diagnosed between January 2007 and December 2008 in Ontario, Canada were identified from the Ontario Cancer Registry. Patient records were linked deterministically to multiple provincial administrative health care databases to provide comprehensive medical follow-up. Exclusion criteria were set to exclude patients on chemotherapy for advanced breast cancer. Any patient with who received at least 1 cycle of adjuvant chemotherapy was included in the analysis. Serious toxicities resulting in emergency room (ER) visits or hospitalizations occurring between the start date of chemotherapy and 30 days after the last dose of chemotherapy were identified. Logistic regression models were used to identify the impact of chemotherapy regimen, age, comorbidity and duration on therapy on the likelihood of experiencing serious toxicity.

Results: Of the 3090 women identified in our cohort, 1440 (46.6%) experienced at least 1 serious toxicity resulting in an ER visit during their adjuvant treatment. Of the ER visits, the majority (1107, 87%) were attributable to treatment-related toxicities. Febrile neutropenia (FN) was the most common treatment-related toxicity occurring in 27.1% of patients in the cohort. Docetaxel containing regimens were associated with a significantly higher rate of ER visits and FN (54.6% vs. 34.6%) compared with paclitaxel (38.0%, 17.9%), or anthracycline alone (epirubicin 45.8%, 23.8%; doxorubicin 32.8%, 15.4%). Table 1 displays the impact of clinical and patient factors on multivariable analysis.

Multivariable analysis for serious toxicity on adjuvant chemotherapy in EBC

<table>
<thead>
<tr>
<th>Regimen Type</th>
<th>Any Acute Care Contact</th>
<th>Hospitalization for Febrile Neutropenia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR</td>
<td>95% CI</td>
</tr>
<tr>
<td>Anthracycline + paclitaxel</td>
<td>1.31</td>
<td>1.01 - 1.71†</td>
</tr>
<tr>
<td>Anthracycline + docetaxel</td>
<td>2.58</td>
<td>2.12 - 3.13†</td>
</tr>
<tr>
<td>Epirubicin Alone</td>
<td>3.57</td>
<td>1.13 - 1.22**</td>
</tr>
<tr>
<td>Doxorubicin Alone</td>
<td>1.00</td>
<td>Ref</td>
</tr>
<tr>
<td>Age (y)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;65</td>
<td>1.00</td>
<td>0.99 - 1.02</td>
</tr>
<tr>
<td>Income Quintile (lowest to highest)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q1</td>
<td>1.32</td>
<td>1.04 - 1.64*</td>
</tr>
<tr>
<td>Q2</td>
<td>1.07</td>
<td>0.85 - 1.34</td>
</tr>
<tr>
<td>Q3</td>
<td>1.15</td>
<td>0.92 - 1.44</td>
</tr>
<tr>
<td>Q4</td>
<td>0.93</td>
<td>0.75 - 1.63</td>
</tr>
<tr>
<td>Q5</td>
<td>1.00</td>
<td>Ref</td>
</tr>
<tr>
<td>Charlson Comorbidity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤3</td>
<td>1.59</td>
<td>1.16 - 2.14**</td>
</tr>
<tr>
<td>&gt;3</td>
<td>1.09</td>
<td>0.90 - 1.32</td>
</tr>
<tr>
<td>Duration on chemotherapy</td>
<td>1.04</td>
<td>1.02 - 1.05†</td>
</tr>
</tbody>
</table>

* P < 0.05; † P < 0.001; ‡ P <0.0001

Conclusion: Serious toxicities are a common in women undergoing adjuvant chemotherapy for EBC and result in significant acute health care utilization.

P5-18-03

First Interim Toxicity Analysis of the Randomized Phase III WSG Plan B Trial Comparing 4xEC-4xDoc Versus 6xTC in Breast Cancer Patients with HER2 Negative Breast Cancer (BC).

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Background: Anthracycline-taxane based adjuvant chemotherapy (cht) is considered standard in node-positive and high-risk node-negative BC. However, retrospective analyses suggest that in HER2-BC, benefit from anthracyclines may not outweigh acute and long term toxicities. Recurrence Score (RS) identifies patients who are not candidates for cht based on their low relapse risk, as well as minimal, if any, benefit of cht. The WSG Plan B trial investigates anthracycline-free cht in HER2-BC and is the first trial in Europe prospectively incorporating RS for decision making regarding adjuvant cht in both N0 and N+ BC.

Methods: Plan B trial randomizes HER2-BC patients with high-risk N0 (at least one risk factor: ≥pT2; negative HR status; ≥23-3 age ≤35 years old; high upPA/PAI-1) or N+ disease to 6xTC (Docetaxel 60mg/m2Cyphosphamide vs. 4xEC (Epirubicin60mg/m2Cyphosphamide) vs. 4xDocetaxel100mg/m2 G-CSF prophylaxis is recommended according to current ASCO guidelines. The statistical design previews n=2.448 randomized to cht; patients with HR+ BC, N0-3 and a RS ≤11 receive endocrine therapy only.

Results: From April 2009 to June 2011, 3037 patients have been recruited and 2296 randomized (TC/EC-Doc: 1146/1150; age <65 years old: 900/911; ≥65 years old: 246/239). From the patients with HR+ disease (n=2368) 18% had a RS 0-11, 61% a RS 12-25 and 21% a RS ≥25. In patients with 0-3 positive LN and RS of 0-11 (n=329) who opted for no cht 257 are in the observational arm. In the group with an intermediate risk (RS 12-25) 14% drop outs before start of cht have been reported. In 1172 fully monitored patients 22 toxicity-related therapy stops have been reported in the TC and 34 in the EC-Doc arm (p=0.12).

614 serious adverse events (SAE) have been reported (299 TC vs. 315 EC-Doc). There is no difference in patients <65 years old (TC vs. EC-Doc: 218/218), but slightly more SAE’s in patients ≥65 years old treated by EC-Doc (97 vs. 81, p=0.13). The most frequent SAEs were: leucopenia, febrile neutropenia (TC/EC-Doc:37 (3.3%)/31 (2.7%), n.s.), infections and heart/vascular events (TC/EC-Doc: 29/40, n.s.). In patients ≥65 years old, there is a trend towards more febrile neutropenia (13 vs. 5; p=0.12).

Detailed data on relationship between the protocol specified, RS-guided treatment assignment and toxicity, and use of G-CSF support will be updated for the meeting.

Conclusions: The Plan-B trial is one of the largest randomized phase III trials currently evaluating anthracycline-free adjuvant cht in HER2-BC. The cht administered within the study was generally well tolerated, but higher number of treatment-related deaths has been observed within the TC arm. The short term toxicity profile seems be different between both study arms, particularly in patients ≥65 years old. On the basis of prognosis as determined by RS, cht has been spared after a shared decision process in a substantial group of patients.

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P5-18-04
Safety Profile of Ixabepilone as Adjuvant Treatment for Poor Prognosis Early Breast Cancer: First Results of the Unicancer-PACS 08 Trial.
Campone M, Spielmann M, Wilders H, Cotta P, Kerbrat P, Levy C, Mayer F, Bachelot T, Winston T, Eymard J-C, Uwer L, Machiels J-P, Verhoeven D, Jaubert D, Facchini T, Orfeuvre H, Canon J-L, Asselain B, Roca L, Lacroix Triki M, Martin AL, Roche H, Centre René Gauducheau, Nantes, France; Institut Gustave Roussy, Villejuif, France; Katholique Universiteit, Leuven, Belgium; Institut Curie, Paris, France; Centre Eugène Marquis, Rennes, France; Centre François Baclesse, Caen, France; Centre Georges-François Leclerc, Dijon, France; Centre Léon Bérard, Lyon, France; Mayo Clinic Florida, Jacksonville; Institut Jean Godinot, Reims, France; Centre Alexis Vautrin, Nancy, France; UCL Cliniques Universitaires SAINT-LUC, Bruxelles, Belgium; AZ Klina Oncology, Brasschaat, Belgium; Clinique Tivoli, Bordeaux, France; Polyclinique de Courfancy, Reims, France; Centre Hospitalier de Fleyriat, Bourg en Bresse, France; Grand Hospital de Charleroi, Charleroi, Belgium; Centre Val d’Aurelle, Montpellier, France; Institut Claudius Regaud, Toulouse, France; R&D Unicancer, Paris, France

Purpose: PACS 01 trial demonstrated that the sequential adjuvant chemotherapy with FEC100 followed by docetaxel (D) significantly improves disease-free and overall survival in node-positive (N+) early breast cancer (BC). However, Triple negative (TN) and ER+/PR-/HER2- subgroups are significantly associated to a worse prognosis even after adjuvant of D. As Ixabepilone (Ixa) has notable preclinical and clinical activity in these subgroups, the PACS 08 trial aims to compare standard FEC100-D regimen to 3 cycles of Ixa followed by 3 cycles of Ixa. We report the preliminary results of the toxicity profile.

Patients and methods: Patients (pts) had localized resectable unilateral ER-/PR-/HER2- or ER+/PR-/HER2- BC. Main inclusion criteria were: age<70 years, normal cardiac, hepatic, haematological and renal functions. Arm A: pts received 3 cycles of FEC100 (F and C, each at 500 mg/m2, E 100 mg/m2, every 3 weeks) followed by 3 cycles of D (100 mg/m2 every 3 weeks); Arm B, Ixa 40 mg/m2 replaced D. Radiotherapy was mandatory after conservative surgery and endocrine therapy was given to ER+ pts. A 5% absolute difference in disease-free survival at 5 years is the main statistical end-point.

Results: Between October 2007 and September 2010, 762 pts with a mean age of 57.9 years (range 30.5 to 83.6 years) were included. Of these, 204 (61.4%) did not receive primary prophylaxis with CSF. Results: 332 patients with a mean age of 57.9 years (range 30.5 to 83.6 years) were included. Of these, 204 (61.4%) did not receive primary prophylaxis with CSF. The true incidence of FN in community based patients may be higher than in the original clinical trial population as non-trial patients may have risk factors known to increase the risk for chemotherapy-induced FN, including increased age or other comorbid conditions. We wanted to know the incidence of FN in patients receiving TC chemotherapy who were not given primary prophylaxis with CSF.

Methods: Using our electronic medical record system, a retrospective review of patients starting TC for breast cancer in 2010 at Kaiser Permanente Northern California was included. Patients had started a four or six cycle regimen of docetaxel 75 mg/m2 and cyclophosphamide 600 mg/m2 every 21 days. Patients were stratified into two groups: (1) CSF primary prophylaxis given with the first cycle versus (2) no CSF primary prophylaxis given with the first cycle. CSF prophylaxis was given by physician choice. FN episodes were defined with a clinical diagnosis code for FN from emergency department visits or hospitalizations. The primary outcome was the incidence of TC-induced FN in patients who did not receive CSF primary prophylaxis with the first cycle of treatment.

Results: 332 patients with a mean age of 57.9 years (range 30.5 to 83.6 years) were included. Of these, 204 (61.4%) did not receive primary prophylaxis with CSF (mean age 57.4 years, range 30.5 to 83.6 years), and 128 (38.6%) received primary CSF prophylaxis (mean age 58.5 years, range 36.6 to 84.2 years). The incidence of FN during any cycle was 24.5% (50/204) in those who did not receive primary CSF prophylaxis and 8.6% (11/128) in those who did (p=0.0003). Patients were hospitalized for FN for a total of 174 days (mean 3.2 days, range 1 to 13 days). Mean days hospitalized were 3.2 in each group. We will present risk factors associated with the development of FN.

Conclusion: In the largest population based report to date, we report the incidence of febrile neutropenia to be 24.5% in patients not
P5-18-06
Taxanes and Cyclophosphamide Are Equally Effective in Breast Cancer: A Meta-Analysis of Ten Phase III Trials in Early and Advanced Disease.

Vriens BE, Lobbezoo DJ, Voogd AC, Veeck J, Tjan-Heijnen VC. Maastricht University Medical Centre, Maastricht, Netherlands; ZonMw, Maastricht, Netherlands

Background: Overall taxanes did not improve survival in metastatic breast cancer trials (n=10 trials), whereas they do so in (neo-)adjuvant breast cancer trials (n=28 trials). Taxanes are widely used but no evidence of survival benefit in metastatic disease. This meta-analysis was conducted to evaluate the efficacy of taxanes and cyclophosphamide in (neo-)adjuvant breast cancer trials.

Methods: We searched MEDLINE, EMBASE, Cochrane Central Register of Controlled Trials, and grey literature databases. Seven meta-analyses were included. Taxane regimens were compared with regimens not containing taxanes (control) or with cyclophosphamide alone. The primary endpoint was disease-free survival (DFS).

Results: In total, 46 studies in early and advanced breast cancer were included and analyzed for their primary endpoint. In metastatic breast cancer studies, the hazard ratio of overall survival was 1.95 (95% CI 1.53 to 2.48) for taxanes compared with cyclophosphamide. In early breast cancer, taxanes showed no improvement for DFS (hazard ratio HR=1.01, 95% CI 0.82 to 1.25). For all studies substituting taxanes for prolonged cyclophosphamide, i.e., AT versus AC, a pooled analysis was performed using RevMan 5 (RevMan 5) providing complete data.

Conclusion: Taxanes were at least as effective as cyclophosphamide in the adjuvant setting. Based on this meta-analysis, taxanes may be considered as an alternative for cyclophosphamide as the first-line agent in hormone therapy-resistant metastatic breast cancer. Further studies are needed to determine if taxanes can replace cyclophosphamide in future trials or in daily practice.

P5-18-07
Presence of Disseminated Tumor Cells after Adjuvant Chemotherapy in Breast Cancer and Disseminated Tumor Cells Monitoring during Secondary Adjuvant Treatment.

Synnestvedt M, Borgen E, Wist E, Wiedswang G, Weyde K, Risberg T, Kersten C, Mjaaland I, Vindi L, Schirmer CB, Nesland JM, Naume B. Oslo University Hospital, The Radium Hospital, Oslo, Norway; Lilleløkks gate, Oslo, Norway; Hospital Innlandet, Gjøvik, Norway; University Hospital Northern Norway, Tromsø, Norway; Sorlandet Hospital Kristiansand, Kristiansand, Norway; Stavanger University Hospital, Stavanger, Norway; Ålesund Hospital, Ålesund, Norway; University of Oslo, Norway

Introduction: Detection of disseminated tumor cells (DTC) after completion of systemic adjuvant treatment is a strong predictor of early systemic relapse and death. This analysis can discover early failure of a chosen adjuvant systemic treatment. In this study, we wanted to evaluate the value of DTC detection in bone marrow (BM) as a surrogate marker for response to docetaxel rescue treatment, to predict the effect of this treatment. Further, we wanted to compare disease-free survival between patients treated with docetaxel resulting in eradication of DTC after treatment and patients treated with docetaxel where DTC persists after treatment. The follow-up of the study is still ongoing. Here, we present the preliminary descriptive data from the study.

Materials and Methods: A total of 1128 pts with node positive or high risk node negative disease (T1c/T2GII-IIIN0) was enrolled in the period from October 2003 to May 2008. All patients had completed primary surgery and received 6 cycles of adjuvant antracycline containing chemotherapy. The first BM aspiration was performed 8-12 weeks after termination of adjuvant chemotherapy (BM1). A second BM aspiration was performed 6 months later (BM2). The processing of BM and DTC analysis (by ICC) were performed as previously described (Wiedswang G et al, J Clin Oncol 2003). If BM2 was positive (+) for DTC, the patient was treated with docetaxel (3qw; 6 courses) D docetaxel-treated patients were reexamined at the inclusion hospital with new BM analysis at approximately 1 month (BM3) and 13 months (BM4) after the last docetaxel infusion.

Results: Of 997 patients with conclusive DTC results for both BM1 and BM2, 83 patients (8.3%) were BM1 positive and 78 (7.8%) BM2 positive. Among the BM1+ patients, 15 (18.1%) were BM2+. The concordance between BM1 and BM2 were 87%. Of the patients positive at one or both time points, the concordance was 10% (15/146). The BM1 was not significantly associated with primary tumor characteristics (although borderline significance for Grade and ER status), whereas for BM2, DTC+ patients had increased frequency of node positive disease and pN2-3 stage (p=0.001, chi-square), and were positively associated with lobular carcinoma (p=0.01, chi-square).

Conclusions: Detection of DTCs after adjuvant antracycline containing chemotherapy changes during the first 9 months of FU, with increased DTC positivity among patients with pN+ disease and lobular carcinomas. After docetaxel rescue treatment, the majority of patients experience disappearance of the DTCs. The clinical significance of these results awaits mature FU data, but the present results may indicate possibility for eradication of residual disease by alternative chemotherapy.

P5-18-08
A Comparative Effectiveness Analysis of Trastuzumab Persistence between Two Adjuvant Breast Cancer Treatment Regimens among US Health Plan Enrollees.

Lalla D, Pelletier EM, Goodman S, Brammer M, Schabert VF. Genentech Inc., South San Francisco, CA; IMS Consulting Group, Watertown, MA; IMS Consulting Group, Woodland Hills, CA

Background: Approved US indications for trastuzumab include adjuvant treatment of HER2-overexpressing breast cancer in anthracycline-based (ACTH) or non-anthracycline-based (TCH) regimens. The objective of this study was to compare trastuzumab persistence across adjuvant breast cancer treatment regimens for US health plan enrollees.
Materials and Methods: This retrospective cohort study using the IMS LifeLink™ Health Plan Claims Database included adjuvant breast cancer patients, defined as those with a mastectomy or lumpectomy and a primary breast cancer diagnosis (index diagnosis) from 1/1/2006 to 12/31/2008. Additional inclusion criteria were: aged ≥18 years; a second primary breast cancer diagnosis ≤90 days of the first; no other primary or secondary neoplasms ≤180 days before or ≤90 days after the index diagnosis; a first trastuzumab claim (index claim) and ≥1 carboplatin, cyclophosphamide, taxane, or anthracycline claim ≤210 days of the index diagnosis; and no trastuzumab claim before the index diagnosis. Regimens were classified as ACTH (doxorubicin, cyclophosphamide, a taxane, trastuzumab) or TCH (docetaxel, carboplatin, trastuzumab) from therapies observed after the index claim. Trastuzumab persistence (days from the index claim to earliest of trastuzumab discontinuation, a new malignant neoplasm, end of plan enrollment, 360 days after the index claim, or end of available data) was reported for patients with ≥2 trastuzumab claims and compared across regimens using the Kaplan-Meier estimator and Cox proportional hazards models.

Results: 550 breast cancer patients met all study criteria (291 ACTH, 259 TCH, 46 unassigned); patients receiving TCH were older (median 52 vs. 50 years; P=0.018). For years 2006-2008, the annual share of the cohort initiating ACTH decreased from 69.3% to 35.2%; patients initiating TCH increased from 26.7% to 56.5%. Patients receiving ACTH were treated with trastuzumab for fewer days (mean=256, median=334) versus patients on TCH (mean=282, median 344, P=0.018). Fewer patients on ACTH remained on trastuzumab at day 90 (83% vs. 91%), day 180 (71% vs. 79%), day 270 (61% vs. 72%), and day 360 (20% vs. 26%). Compared to patients on TCH, patients on ACTH continued to be treated with trastuzumab for a shorter duration post-index after adjusting for age, region, plan type, prescriber specialty, and comorbidities (HR=0.77, 95% CI [0.63, 0.95]; P=0.016).

Discussion: The share of adjuvant breast cancer patients initiating TCH increased from 2006 to 2008, while use of ACTH decreased over the same time period, and patients treated with TCH remained on trastuzumab for a longer period of time. From a clinical standpoint, patients derive maximum benefit from their medications when they are persistent long-term, which can lead to economic benefits with regards to lower overall healthcare costs. Further analyses will evaluate whether adjuvant breast cancer regimens differ in weight-adjusted cumulative trastuzumab doses received during therapy.

P5-18-09

The Incidence of Febrile Neutropenia in the First Course of Adjuvant Chemotherapy with Docetaxel/Cyclophosphamide with or without Pegfilgrastim.

Jones S, Paul D, Sedlacek S, Vukelja S, Wilks ST, Stokoe C, Osborne CR, Krekow L, McIntyre K, Holmes FA, Guerra L, Zhan F, Asmar L, O’Shaughnessy J, Blum JL. US Oncology, The Woodlands, TX; Baylor-Sammons Cancer Center, Baylor University, Dallas, TX; Rocky Mountain Cancer Center; Denver, CO; Texas Oncology-Tyler, Tyler, TX; Cancer Care Centers of South Texas, San Antonio, TX; Texas Oncology, Plano, TX; Breast Cancer Center of North Texas, Bedford, TX; Texas Oncology-Dallas Presbyterian Hospital, Dallas, TX; Texas Oncology-Houston Memorial City, Houston, TX

Background: In our original doxorubicin-cyclophosphamide/docetaxel-cyclophosphamide (AC/TC) adjuvant study (JCO 27: 1177-1183, 2009), we reported an incidence of febrile neutropenia (FN) of 5% (8% in women ≥65 years) with the TC regimen without prophylactic WBC growth factors but with a recommendation for prophylactic antibiotics. There is a paucity of data on the incidence of FN with the TC regimen aside from this clinical trial. Because we have been conducting a randomized adjuvant study of TC compared to other regimens, we used this opportunity to analyze the incidence of FN during the first course of chemotherapy with TC in the first cohort of randomized patients (US Oncology Network study 06090). The prophylactic use of WBC growth factors was at the investigator’s discretion.

Patients and Methods: The study included 1298 patients entered between May 2007 and May 2009. Of these, 649 were included in the TC arm. Median age was 54 years (range 27-71), 75.5% were Caucasian, 561 (86.4%) were in PS 0 at baseline, and about half were node negative. Eight patients did not receive study treatment for various reasons. Among the 641 patients who received TC; 213 (33.3%) received pegfilgrastim, 48 (7.5%) received filgrastim and were not included in this analysis, and 380 (59.2%) patients did not receive either during the first cycle. Thus, this analysis focused on 593 women who did or did not receive prophylactic pegfilgrastim in cycle 1.

Results: All patients with a reported adverse event of FN or with a reported AE of fever with some degree of neutropenia (in order to capture all possible cases of FN) during the first cycle of TC were identified [Table 1]. FN and fever + neutropenia occurred in a total of 6 (2.8%) patients who received pegfilgrastim and 36 (9.5%) patients who did not. A comparison of age, race, performance status and stage of disease between these 2 groups revealed that they were similar. The 213 patients who received pegfilgrastim were slightly older (median 56 years, range 27-71) compared to those who did not (median 53 years, range 30-71).

| FN and Fever+Neutropenia During Cycle 1
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<td>FN</td>
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During all 6 cycles, 41 patients reported FN, and 30 (73%) of these patients experienced FN during cycle 1.

Conclusion: Among 593 women who received TC as adjuvant chemotherapy, the incidence of FN during the first cycle was under 10% whether or not the patients received prophylactic pegfilgrastim.

P5-18-10


Chan A, McGregor S. Mount Hospital, Perth, WA, Australia

Background: Chemotherapy for early breast cancer (EBC) confers significant survival advantages. However, myelosuppression is a common cause of chemotherapy dose reduction that may subsequently compromise survival benefits. Primary prophylaxis (PG) with granulocyte-colony stimulating factor (G) is indicated for TAC regimen, but rates of grade 3/4 neutropenia (G3/4N) associated with other adjuvant regimens in a non-trial setting are not well documented. Optimal use of G will likely enable planned dose delivery, leading to optimal BC outcomes. This retrospective study reports dose delivery outcome with judicious use of G in a single institution.

Methods: Consecutive patients (pts) with EBC who received chemotherapy at Mount Hospital from January 1999 - December 2010 were included. Data was collected prospectively for pt characteristics, regimen given and neutropenic complications. Only pts who received a minimum of 1 cycle of chemotherapy were included. PG
was only available in pts receiving TAC after 1/4/2007; Secondary G propha	xlsis (SG) was given for G3/4N in first cycle, febrile neutropenia (FN) or delay in neutrophil recovery (DN). Primary outcome measure was dose delivery defined as relative dose intensity (RDI - fraction of dose per unit of time of standard regimen) and fraction planned dose received (PDR). Secondary endpoints included EBC outcome and incidence of haematological malignancies.

Results: Over the 12 year period, 1655 pts (62% node positive) with mean age of 51yr (24-88yr) were included. Chemotherapy given - n (%): Non-anthracycline(A)/taxane(T) 63 (3.8); A 671(40.5); AT 666(40.2); T-only 258(15.4). Overall 64% of pts received G; PG given for TAC in 167(10); SG was given for first cycle G3/4N 677(41), FN 87(5) - 89% during first cycle, DN 109(7). Mean RDI and PDR was 0.97 v 0.95 and 0.98 v 0.97 for pts who received G and did not, respectively. Amongst pts aged < or ≥65yr, rate of G use and >85% PDR were comparable (65% v 66%; and 4% v 6%, respectively). Five cases of myelosuplasia or acute leukemia were reported (4 pts received G) -0.37% v 0.17% of pts having G or not, respectively. All cases received an anthracycline with cyclophosphamide. At median follow-up of 44.9 months [Q1: 25.1- Q3: 69.6], DFS was 88.9% and OS 92.6%. PDR <85% occurred in 5% pts, with OS of 78% v 93% if pts received < or ≥85%, respectively.

Discussion: Significant rates of myelosuppression occur with regimens commonly used in the community. These regimens are not associated with FN rates >20% and thus PG is not advised. Routine monitoring of first cycle neutrophil nadir enabled the identification of those pts at high risk of developing further neutropenic complications and consequent need for dose reduction or delay. Utilisation of G in these high risk pts enabled a high RDI to be delivered, comparable to pts who did not experience significant myelosuppression. Further, pts who did not develop significant first cycle myelosuppression were largely spared the need for G. In this manner, concerns regarding haematological toxicity and costs associated with G use are minimised.

P5-18-11 Incidence of Chemotherapy Dose Reductions and Dose Delays, and Reduced Chemotherapy Dose Intensity in Early Stage Breast Cancer.

Weckler D, Edelsberg J, Kartashov A, Barron B, Lyman G. Policy Analysis Inc. (PAI), Brookline, MA; Amgen Inc., Thousand Oaks, CA; Duke University, Durham, NC

Background: Chemotherapy is widely used to treat early stage breast cancer (ESBC). Dose reductions and dose delays--e.g., due to advanced age or severe/febrile neutropenia--are generally believed to increase risk of disease progression and reduce survival. Little is known about incidence of chemotherapy dose reductions and dose delays among women with ESBC in clinical practice.

Methods: This study employed a retrospective cohort design and electronic medical records from over 60 community oncology clinics in more than 20 states (2004-2010). Study population included adult women who received myelosuppressive chemotherapy for ESBC (stages I-IIIA). For each such woman, each unique cycle of chemotherapy within their first observed course was identified. Incidences of chemotherapy dose delays (≥7 days for any drug in ≥1 cycles), chemotherapy dose reductions (≥15% for any drug in ≥1 cycles), and low relative chemotherapy dose intensity (<85% over the course) -- relative to physician-reported planned chemotherapy administration -- were descriptively analyzed for the seven most frequently observed regimens among subjects.

Results: 2,350 women received seven most frequently observed regimens (80% of study population); mean age -- across regimens -- ranged from 51-56 years, and 31-94% received primary prophylaxis against severe/febrile neutropenia with a colony-stimulating factor. Incidence of dose reductions ranged from 17-48%, and dose delays, from 24-45%. Overall mean relative dose intensity (RDI) ranged from 80%-99%, and 17-50% of subjects received RDI <85%; mean RDI among those with low RDI ranged from 40%-67%.

Discussion: Chemotherapy dose delays and dose reductions are common in ESBC, among those receiving dose-dense as well as conventional regimens.
higher with TCH. For example, there were fewer dose modifications/delays for AC-TH than TCH (31% vs. 46%, p=0.07). This may have been due to common use of GCSF with AC-TH (77% vs. 33% use with TCH). Neutropenic fever (NF) was higher with TCH, reaching 25% incidence when administered without GCSF. However, NF did not occur in the 8 TCH patients who received cycle 1 GCSF. There was no correlation between NF and patient age. The incidence of left ventricular EF decline leading to cessation of trastuzumab was similar for both regimens (19.4% AC-TH vs. 14.6% TCH; p = 0.64). Trastuzumab was completed as planned in 70% of patients. Although EF decline was most common explanation, 13% of early trastuzumab discontinuations occurred for other reasons.

CONCLUSION: TCH and AC-TH are the most commonly administered adjuvant regimens for WI women with HER2+ BrCa. Amongst WI oncologists, TCH is perceived as safer, but is less likely to be recommended for node-positive BrCa. This retrospective analysis suggests that acute myelosuppression is greater for TCH, with a significant rate of NF. Per ASCO guidelines, these data suggest GCSF should be used routinely with TCH due to high rate of FN.

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P5-18-13
Adjuvant Chemotherapy with Vinorelbine+5FU or Capecitabine in Poor Responders to Neoadjuvant EC-Docetaxel Chemotherapy (NAC) for Locally Advanced Breast Cancers.


Background: Post-operative chemotherapy in poor responders after NAC is questionable. We evaluated the use of adjuvant Vinorelbine (NVB) + continuous infusion 5FU or Capecitabine (Cap) in women with poor pathological response to preoperative sequential Epirubicin Cyclophosphamide-Docetaxel (ECT).

Materials and methods: From May 2004 till October 2007, 340 patients (pts) with stage II or III breast adenocarcinoma received neoadjuvant ECT +/- Trastuzumab (HER2+), +/- Celecoxib (HER2 negative) in a randomized phase II trial (Pierga, 2010). 53 patients (15.5%) had pathological complete response (no infiltrative or in situ carcinoma in the breast and lymph nodes). Eleven pts were not operated. 276 pts had grade 3-4 Chevallier residual disease. These poor responders could be optionally offered post surgery chemotherapy (NVB+5FU or Cap)+/- Trastuzumab. This regimen started concomitantly with post-operative RT. All positive hormonal receptors received hormone therapy. We attempted to evaluate DFS according to post operative CT in the 276 pts. To take into account the differences between populations, treated and untreated patients were matched (1:1) according to variables with a significant difference between the two groups, analyses were adjusted with all other prognostic factors.

Results: Out of 276 pts with 3-4 Chevallier’s grade after NAC, 198 pts did not receive any CT (group A), 78 received adjuvant CT (group B); NVB + 5-FU (FUN: N=41) or Cap (N=17) + Trastuzumab (21pts, 11 FUN and 10 CapNVB), 28 pts HER2+ in the group A receive Trastuzumab alone after surgery. The main characteristics of these patients differed for the initial lymph nodes status (N0: 15 (19 %) in group A vs 88 (44 %) in group B, p = 0.0001 and the number of positive lymph nodes after surgery (mean: 4.39 vs 1.95, p = 0.0004 respectively in groups A and B).

With a median follow up of 38.9 months, 63/276 pts (22.8%) recurred, 44 (22 %) in the group A and 19 (24.3 %) in the group B. DFS for the 276 pts is 95% at 12 months (m) and 86% at 24 m, 95% and 87% in group A and 96% and 83% in group B (logrank test : p = 0.63). We matched the 2 groups (124 pts; 62 [group] according to initial lymph nodes status, local response (Chevallier grade 3 or 4) and pN status after NAC. Characteristics of this matched population were similar except for more ER negative in chemo group, 19 pts (30.6 %) and 9 pts (14.5 %) in non chemo arm. In this population, 27 events occurred, with 13 in the chemo group and 14 in the non chemo’one. Treatment effect was non significant in univariate analysis (p = 0.93).

In multivariate analysis adjusted for initial tumor size (TNM), age, molecular status (hormonal receptor and HER2 status), histological grade, initial lymph node status, histological response after NAC, treatment effect was non significant (Hazard Ratio = 1 IC95% [0.38 ; 2.67]; p = 0.99). There was no difference in treatment effect according to molecular status (test for interaction p = 0.30). This multivariate analysis was also completed in the 276 pts with similar results.

Conclusion: In poor responders patients after Anthracyclins-Taxanes NAC, adjuvant chemotherapy with Vinorelbine- 5-FU or Capecitabine- did not appear to prolong DFS.

P5-19-01

Campone M, Dobrovolskaya N, Tjulandin S, Chen S-C, Fourie S, Mefti F, Konstantinova M, Lejresne F, Meheust N, Jasson J. Institut de Cancérologie de l’Ouest/René Gauducheau, Saint Herblain, Nantes Cedex, France; Russian Research Center of Roentgenoradiology, Moscow, Russian Federation; Russian Oncological Research Center, Moscow, Russian Federation; Chang Gung Memorial Hospital, Taichung, Taiwan; Wilmed Park Oncology, Klerksdorp, South Africa; Centre René Huguenin, Saint-Cloud, France; Moscow Regional Oncology Dispensary, Moscow, Russian Federation; Institut de Recherche Pierre Fabre, Boulogne, France; Medical University of Gdansk, Gdansk, Poland

Purpose: Owing to the increasing number of patients treated with anthracycline-based adjuvant chemotherapy, there is a need for new effective and tolerable non-anthracycline based regimens in metastatic breast cancer.

Patients and methods: Patients with HER2-negative metastatic breast cancer previously treated with anthracyclines in (neo) adjuvant setting were randomised to fully oral 3-weekly cycles of the combination of oral vinorelbine with capcitabine (V+C), to the same drugs alternating every 3 cycles (V+C), or to the combination of docetaxel and capcitabine (D+C). V was given at 80 mg/m² (after the first cycle at 60 mg/m²) on days 1 and 8 in the V+C arm and weekly in the V+C arm, 100 mg/m² bid from days 1 to 14, and D on day 1 at 75 mg/m². The primary endpoint was disease control rate (CR+PR+NC ≥ 3 months).

Results: A total of 139 patients were randomly assigned to V+C (44 patients), V+C or D+C (47 patients) and D+C (48 patients). After an independent review, the disease control rate in the intent-to-treat population in the V+C, V+C and D+C arms (95% CI) was 70.5% [54.8-83.2], 37.0% [23.2-52.5] and 70.8% [55.9-83.1]. The response
rate was 31.8% [18.6-47.6], 8.7% [2.4-20.8] and 35.4% [22.2-50.5], respectively. The median duration of progression-free survival in the V+C, V=C and D+C arms [95% CI] was 7.2 months [5.3-8.9], 3.4 months [2.6-5.6] and 8.9 months [7.2-12.0]; the median overall survival [95% CI] was 22.2 [18.8; 29.9], 19.4 [12.5; 35.4] and 24.2 [14.2; 38.5] months, respectively. Lower efficacy observed in the sequential arm could be due to higher prevalence of patients with visceral disease in this arm (91.3% in comparison to 65.9% in V+C and 64.6% in D+C arm). Combinations of V+C or D+C showed similar efficacy and a different toxicity profile; V+C induced less neutropenia, infection, hand-foot syndrome, fatigue/asthenia and alopecia, whereas D+C- less gastrointestinal toxicity.

Conclusions: V+C combination constitutes a valuable fully oral alternative option to D+C in patients with metastatic breast cancer previously treated with anthracyclines in (neo)adjuvant setting, while offering the advantages of an all-oral treatment.

P5-19-02
Comparison of the Incidence of Peripheral Neuropathy with Eribulin Mesylate Versus Ixabepilone in Metastatic Breast Cancer Patients: A Randomized Phase II Study.
Vahdat L, Gopalakrishna P, Garcia AA, Vogel C, Pellegrino C, Lindquist D, Iannotti N. Weill Cornell Medical College, New York, NY; Eisai, Inc., Woodcliff Lake, NJ; University of Southern California Keck School of Medicine, Los Angeles, CA; Sylvester Comprehensive Cancer Center, University of Miami, Miller School of Medicine, Deerfield Beach, FL; Montefiore Medical Center, New York, NY; US Oncology, Sedona, AZ; Hematology-Oncology Associates of the Treasure Coast, Port Saint Lucie, FL

Background: Peripheral neuropathy (PN) is a common toxicity and treatment limiting factor in heavily pretreated patients (pts) with metastatic breast cancer (MBC). Consequently, tolerability is important when choosing treatment. Eribulin mesylate has recently been approved in the US for patients with MBC who have previously received at least two chemotherapeutic regimens for metastatic disease including an anthracycline and a taxane. Eribulin was associated with a 34.6% incidence of PN (7.8% grade 3; 0.4% grade 4), however, over half of pts experiencing grade 3/4 PN continued eribulin. Ixabepilone (IXA) is frequently used in late-line MBC pts and registration studies reported an overall PN incidence of 63% (14-21% grade 3/4). We sought to define incidence of PN of eribulin and IXA in a late-line setting since indirect comparisons suggest eribulin may have a more favorable neurotoxicity profile compared with IXA.

Methods: This randomized, two-arm, multicenter, open-label, phase II study stratified pts by pre-existing neuropathy (grade 0 or 1) and number of prior chemotherapy (CT) regimens (≤3 or >3). 104 pts with locally recurrent or MBC, who had received at least one prior cytotoxic CT, including a taxane for MBC were randomized to receive eribulin mesylate (n=52; 1.4 mg/m² 2- to 5-min IV on Days 1 and 8 of a 21-day cycle) or IXA (n=52; 40 mg/m² as a 3 h IV infusion on Day 1 of a 21-day cycle). The primary objective was to compare the incidence of neuropathic adverse events. Secondary objectives were: 1) to compare the severity of neuropathy using CTCAE grading, a patient-reported neurotoxicity questionnaire (SRNO), and vibration sensitivity (VS); 2) to evaluate efficacy as measured by objective response rate (ORR), clinical benefit rate (CBR), disease control rate (DCR), and progression-free survival (PFS); and 3) to evaluate safety.

Results: Of 51 eribulin and 50 IXA pts who received treatment, eribulin-treated pts experienced a trend towards less PN and treatment-emergent neuropathy (TEN) than IXA-treated pts (31.4% vs 44.0%, p=0.1632; and 33.3% vs 48.0%, p=0.1284, respectively).

The incidence of grade ≥3 neuropathy was 9.8% and 22% for eribulin and IXA, respectively. Time to onset of severe neuropathy was earlier for IXA relative to eribulin (4.8 vs 9.9 weeks, respectively); by cycle 4, 44% of subjects receiving IXA experienced TEN compared with 24% on eribulin. SRNO and VS results will be analyzed separately. Treatment-emergent adverse events (TEAEs) and serious TEAEs were comparable (98% and 96%; 37% and 34% in eribulin- and IXA-treated subjects, respectively). Eribulin-treated pts experienced less discontinuation of treatment due to neuropathy (3.9% vs 18%) or TEAEs in general (11.8% vs 32.0%) relative to IXA pts. The efficacy data for eribulin vs IXA was 15.4% vs 5.8% for ORR, 26.9% vs 19.2% for CBR, 67.3% vs 55.8% for DCR, and 104 days vs 95 days for median PFS, respectively.

Conclusions: Although the difference in overall incidence of PN and TEN was not significant for eribulin vs IXA, eribulin tended to show less PN and TEN. Eribulin also had less severe and longer time to development of grade 3/4 PN and fewer discontinuations due to toxicity.
Support: Eisai Inc.

P5-19-03
Gradishar WJ, Krasnojon D, Chepovor S, Makhson AN, Manikhas GM, Clawson A, Bhar P. Northwestern University, Chicago, IL; Leningrad Regional Oncology Center; Russian Federation; Yaroslavl Regional Clinical Oncology Hospital; Yaroslavl, Russian Federation; City Oncology Hospital, Moscow, Russian Federation; St. Petersburg Oncology Center, St. Petersburg, Russian Federation; Celgene Corporation, Summit, NJ

Background: We previously reported the results of a phase II study evaluating the efficacy and safety of 3 different dosing regimens of ab-pac and docetaxel for the first-line treatment (Tx) of MBC (Gradishar et al. J Clin Oncol. 2009;27:3611-3619). Here, we report final overall survival (OS) and an analysis of safety and associated dose reductions (DRs). Methods: Patients (pts; N = 300) with previously untreated MBC were randomized to 1 of 4 Tx arms (table). A step-down statistical approach was used for pairwise comparisons of Tx arms. The trial was powered for antitumor activity and safety. Results: Tx arm C produced the longest OS (33.8 months) with an 11.6-month longer median OS vs arm B (HR 0.575; P = .008) and a 7.2-month longer median OS vs arm D (HR 0.688; P not statistically significant). OS data were consistent with previously reported investigator assessment of overall response rates and progression-free survival. Grade (gr) 4 neutropenia (np) was significantly less frequent in the ab-pac arms vs. the docetaxel arm (5-9% vs. 75%); Febrile np occurred in 1% of each ab-pac arm vs 8% in the docetaxel arm. Rates of gr 3 sensory neuropathy (SN) were 21%, 9%, 22% and 12%, respectively, in arms A-D (P = .083). No gr 4 SN occurred. Median time to improvement to ≤ gr 2 SN was 20-22 days in the ab-pac arms vs 41 days in the docetaxel arm. Gr 3 fatigue occurred in 5, 0, 4, and 19% of pts in arms A-D, respectively. In arm C, best response was observed at cycle 2, whereas DRs due to toxicity occurred later, at cycle 4 (table). The percentage of pts dose reduced due to ≥ 1 Tx-related toxicity were 18%, 17%, 47%, and 28% in arms A-D. The median cycles at which DRs occurred were 7, 5, 4, and 3, respectively. Np and SN were the most common toxicities leading to DRs.
Conclusions: Although it is a small sample size phase II study, it strongly suggested that cisplatin-contained TP regimen improved patient outcome, as measured by ORR, CBR, PFS and OS, compared with non-cisplatin-based TX regimen in the first-line treatment of advanced TNBC patients. This is the first prospective study to show superiority of cisplatin over capecitabine in TNBC. Together with additional ongoing clinical trials comparing platinum with other chemotherapeutic agents in various settings, the role of platinum in TNBC can be further elucidated.

P5-19-05
Age-Related Changes in the Pharmacokinetics (pK), Response, and Toxicity of Weekly nab-Paclitaxel in Patients with Metastatic Breast Cancer (MBC).

Background: Although cancer is a disease of aging, few studies have evaluated the association between patient age and the pK or pharmacodynamics (pD) of cancer therapeutics. The goals of this study were 1) to evaluate the age-related changes in the pK and pD of weekly nab-paclitaxel in patients with MBC, 2) to determine response rate; and 3) to explore the relationship of age with pK and pD parameters (i.e., dose reductions, dose delays and grade ≥ 3 toxicities).

Patients and Methods: Forty patients with MBC, receiving 1st or 2nd-line chemotherapy, entered an IRB approved protocol to evaluate the age-related changes in the pK of nab-paclitaxel administered at 100 mg/m² IV for 3 weeks followed by a 1-week break. Patients were accrued from 4 age strata <50, 50-60, 60-70, and >70 years of age. Blood samples were collected for pK analysis with the first dose of nab-paclitaxel administered.

Results: Of the 40 patients who entered the study, 39 (98%) were evaluable with a mean age of 60 (SD=13.4; min=30; max=81). Patients were accrued in the following age cohorts: <50 (n=10; 26%), 50-60 (n=5; 13%), 60-70 (n=15; 38%), and >70 (n=9; 23%) years of age. The median number of courses completed was 4 (min=1, max=21). The response rate was: 0% (n=0) CR, 31% (n=12) PR, 38% (n=15) SD. Grade 3 toxicity was experienced by 26% (n=10). We observed 8% (n=3) grade 3 hematological toxicities [neutrophils (n=1; 3%), leukocytes (n=2; 5%)] and 18% (n=7) grade 3 non-hematological toxicities [nausea and hypophosphatemia (n=1; 3%), diarrhea and infection without neutropenia (n=1; 3%), fatigue (n=2; 5%), hyponatremia (n=1; 3%), and infections without neutropenia (n=2; 5%)]. There were no cases of grade 4 or 5 toxicity. Grade 2 sensory neuropathy was experienced by 8% (n=3; no cases in the 70+ age cohort). Dose reductions or course delays were experienced by 62% (n=24) and 21% (n=8), respectively. There was a borderline significant positive association between age and natural logarithm of

<table>
<thead>
<tr>
<th>Age (years)</th>
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<th>[95% CI]</th>
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<tr>
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P-values: P<0.05; C=control group; T=treatment group; OS=overall survival; PFS=progression free survival; ORR=overall response rate; CBR=clinical benefit rate; PFS=progression free survival; OS=overall survival; ORR=overall response rate; CBR=clinical benefit rate; 5-week nab-paclitaxel regimen.
Background: Triple-negative breast cancer (TNBC) contributes to poor prognosis and there is no established standard chemotherapy this subtype of patients. It has been postulated that related cancers would confer sensitivity to certain cytotoxic agents like cisplatin. Cisplatin and gemcitabine have single-agent activity in metastatic breast cancer and preclinical data support synergy of the combination. The objective of this study was to evaluate doublet with gemcitabine/Cisplatin (GP) as first-line therapy in patients with metastatic TNBC.

Material and Methods: This is a prospective single-institutional, open-label, phase II trial. The primary endpoint was progression free survival (PFS). Eligible subjects were aging from 18 to 75 years old, with no prior chemotherpay for MBC, with tumors negative for ER, PR or HER2, with at least one measurable disease according to the RECIST criteria, with EOCG PS of 0-1, and with adequate organ function. All patients received 21-day cycles of gemcitabine 1,000mg/m² on days 1 and 8 and cisplatin 25 mg/m² on days 1 through 3. Treatment was continued until disease progression or unacceptable toxicity or up to 8 cycles. (ClinicalTrials.gov number, NCT00601159)

Results: A median age was 49 years (range: 29-74 years). 58 patients had received neo/adjuvant chemotherapy (53 patients with anthracycline and/or taxane). The median number of treatment cycles was six (range: 2-8 cycles). In 64 assessable patients, we observed 10 complete response (CR; 15.6%), 30 partial responses (PR; 46.9%), 17 patients (26.6%) with stable disease (SD), and 7 patients (10.9%) with progressive disease, for an overall response rate of 62.5% (95% CI, 50.3% to 74.7%). The median PFS was 7.7 months (95% CI, 6.0 to 9.4). With a median follow-up time of 28 months, the median overall survival was 19.1 months (95% CI, 12.5 to 25.7). Grade 3 or 4 toxicities were neutropenia 42.2%, thrombocytopenia 29.7%, anemia 18.8%, nausea/vomiting 15.6%, fatigue 14.1%, constipation 3%, sensory neuropathy 1.6%, 2 patients developed febrile neutropenia. The chemotherapy doses were reduced in 13.3% (6 pts) because of toxicity. There were no treatment-related deaths.

Conclusion: Significant activity and favorable toxicity profile of GP regimen as first-line chemotherapy in patients with metastatic TNBC. It provided a basis for considering GP for further evaluation in phase III trials for women with metastatic TNBC in China. (ClinicalTrials.gov number, NCT01287624)
Median age: 55 years (range 23–90). Most pts were Caucasian (81%) with ECOG PS of 0 (55%). Baseline demographics were balanced across treatment lines. Data on A/T treatment history were available for 719 pts; 557 pts received prior A/T therapy, 176 were A-refractory, 234 T-refractory and 154 A- and T-refractory. Pooled overall response rate (ORR) was 21.0% (1.7% complete; 19.3% partial). ORR was 25.0% and 19.0% for pts treated in the 1st- and 2nd-line settings (OR 0.70, 95% CI: 0.5–1.0; p=0.0486). Median PFS was 126.9 days (95% CI: 119–132) in the pooled analysis, and significantly longer in pts receiving 1st- vs ≥2nd-line C: 150.0 vs 112.0 days (HR 1.45, 95% CI: 1.23–1.71; p<0.0001). Pooled median OS was 482.1 days (95% CI: 438–516). Significantly longer median OS was seen in pts receiving 1st- vs ≥2nd-line C: 666.0 vs 396.0 days (HR 1.98, 95% CI: 1.62–2.41; p<0.0001). 62 pts (8%) withdrew due to C-related AEs, which were severe in 4% of pts and life threatening in 2%. AEs of special interest occurred in 489 pts (63%); these were severe in 24% of pts and life threatening in 2%. Gastrointestinal disorders were most frequent (417 pts [53%]), and were severe or life threatening in 13% and 1% of pts.

Conclusions: This pooled analysis of pt data (n=805) revealed significantly longer ORR/survival in pts receiving 1st- vs ≥2nd-line C for A-/T-pretreated MBC. Univariate and multivariate analyses are ongoing; results will be presented. Safety data were in-line with findings from phase III trials of C in MBC.

### P5-19-09

**Phase II Trial of Ixabepilone Plus Carboplatin in Patients with Metastatic Breast Cancer: The ECLIPSE Study.**

*Osborne C, Challagulla JD, Fanning SR, Eisenbeis CF, Holmes FA, Monaghan GG, Neubauer MA, Rabe AC, Raja V, Robbins GJ, Taboada C, Vikela SJ, Wilks SF, Wang Y, Brown J-A, Asmar L, O’Sheaughnessy J. US Oncology, The Woodlands, TX; Baylor-Sammons Cancer Center; Dallas, TX; Texas Oncology, Witchita Falls, KS; Cancer Centers of The Carolinas, Greensboro, SC; Cancer Centers of North Carolina, Raleigh, NC; Texas Oncology-Houston Memorial City, Houston, TX; Texas City Cancer Center, Kansas City, MO; Kansas City Cancer Center-Southwest, Overland Park, KS; Kansas City Cancer Center, Kansas City, KS; Florida Cancer Institute-New Hope, New Port Richey, FL; Texas Oncology-Methodist Charlton Cancer Center, Dallas, TX; Texas Oncology-Tyler, Tyler, TX; Cancer Centers of South Texas, San Antonio, TX*

**Introduction:** Ixabepilone adds to the antitumor effectiveness of capecitabine in both ER+ and triple negative (TN) breast cancer. Ixabepilone has antitumor activity in taxane-refractory patients, and novel combinations are needed in this poor prognosis population. Carboplatin combined with gemcitabine or paclitaxel has activity in metastatic breast cancer; there is also demonstrated activity of the gemcitabine/carboplatin combination in the ER+ versus TN subsets. A Phase I study of ixabepilone + carboplatin in solid tumor patients demonstrated the safety of this combination.1 We conducted a Phase II trial of ixabepilone + carboplatin at the doses and schedule used in the Phase I trial to determine its effectiveness in hormone receptor positive (HR+) and TN patients.

**Methods:** This was a Phase II, open label, nonrandomized parallel, noncomparative study of 2 groups. Patients could have received up to 2 lines of treatment for metastatic disease. All patients received ixabepilone 20 mg/m² on Days 1 and 8 and carboplatin AUC=2.5 on Days 1 and 8 of each 21-day cycle. Patients were stratified as either (HR+) (n=50) or (TN) ER−/PR−/HER2− (n=53). Patients received drug until PD or intolerable toxicity. Patients continued treatment for as long as they responded (CR, PR, or SD). The primary objective was to evaluate objective response rate (ORR). Secondary objectives included evaluation of clinical benefit rate (CBR) defined as ORR (CR+PR)+SD≥6 months, progression-free survival (PFS), overall survival (OS), duration of responses, and toxicity.

**Results:** Based on preliminary data, 96 patients (55 [57.3%] HR+ and 40 [41.7%] TN) received study treatment, with 1 patient found ineligible. Median age was 55.2 years; all were females; baseline PS of 0/1/2 was 50/44/2; stage at diagnosis I/II/III/IV/unknown was 12/38/21/2/1, respectively. PR was 26.3% overall. There were no CRs. CBR overall was 41.1% (39), 52.7% (29) for HR+ patients, and 25.6% (10) for TN patients. Median PFS was 7.6 months, and median OS was 12.7 months. The median time to response and duration of response in patients achieving a PR was 1.7 and 6.5 months, respectively. Grade 3/4 hematological toxicities included 49% neutropenia, 10.4% anemia, and 4.2% thrombocytopenia. The most common grade 3/4 non-hematologic toxicities included fatigue (8.3%), nausea (6.3%), neuropathy (6.3%), vomiting (5.2%), and dehydration (5.2%). Of 32 deaths during the study, one was due to neutropenic sepsis, and 25 were due to PD.

**Conclusion:** The combination of ixabepilone plus carboplatin was a tolerable and active regimen for both HR+ and TN breast cancer. In general, it had expected hematologic and manageable non-hematologic toxicities. Further follow-up of the remaining 12 patients still on treatment is ongoing. 1. Plummer R, et al. Clin Cancer Res. 2008 Dec 15;14(24):8288-8294.

### P5-19-10

**Correlation of Response of Weekly Paclitaxel and Paraplatin as First Line Treatment of Metastatic Breast Cancer with p53 Status.**

*El-Sadda W, Magdy M, Abdel-Halim I, Abdel-Wahab A. Mansoura University Hospital, Mansoura, Egypt*

**Background:** Paclitaxel is one of the most active drugs in metastatic breast cancer. Weekly paclitaxel seems less toxic & more efficient compared with paclitaxel every 3 weeks (possibly because of the proapoptotic and antiangiogenic activity), the dose intensity is quiet higher with tolerable toxicity. The sensitivity of tumors overexpressing p53 to paclitaxel is a matter of debate.

The purpose of study is to evaluate weekly paclitaxel and paraplatin in patients with metastatic breast cancer in terms of response rate, toxicity profile, relation of response to p53 status, progression free survival & overall survival.

**Patients & Methods:** Between September 2006 & September 2008, 60 patients with measurable metastatic breast cancer after adjuvant anthracycline treatment, WHO PS <2, adequate renal, liver & bone marrow function. Patients received no prior chemotherapy for metastasis. Pathology specimens were evaluated for p53 status by immunohistochemistry (IHC). All patients received weekly paclitaxel 80 mg/m2 D1, D8, D15 (one hour IV infusion) and paraplatin AUC 5 D1 (IV infusion over
Tesetaxel, an Oral Taxane, as First-Line Therapy for Women with Metastatic Breast Cancer.

Schwartzberg L, Rubin P, Patnaik A, Itri L, Olson AL, Seidman AD. The West Clinic, Memphis, TN; The Moses H. Cone Regional Cancer Center, Greensboro, NC; START Center - South Texas Accelerated Research Therapeutics, San Antonio, TX; Genta Incorporated, Berkeley Heights, NJ; Memorial Sloan-Kettering Cancer Center, New York, NY

Background: Parenteral taxanes (docetaxel, paclitaxel) are among the most active agents in treating metastatic breast cancer (MBC). However, their use is limited by inherent or acquired multidrug resistance, hypersensitivity, and neurotoxicity. Tesetaxel is an advanced-generation, orally available taxane that is formulated as a capsule for oral administration and has a long terminal half-life in plasma (~180 hrs). Unlike standard taxanes, tesetaxel is not a substrate for P-glycoprotein (P-gp), a major cause of taxane resistance. The drug is highly concentrated in cells that overexpress P-gp. In taxane-resistant breast cancer xenografts (DU4475), tesetaxel induced a 94% reduction in tumor size, substantially exceeding the activity of docetaxel and paclitaxel (46% and 26%, respectively). Neurotoxicity was also substantially lower with tesetaxel compared with equivalent doses of docetaxel. Among more than 350 patients (pts), there have been no occurrences of hypersensitivity reactions. An initial phase 2 study as 2nd-line therapy for pts with MBC who progressed after multidrug anthracycline-containing regimens showed a 38% partial response (PR) rate using tesetaxel Q3 wks at a dose of 27-35 mg/m². We conducted a Phase 2, open-label, multicenter study of the efficacy and safety of tesetaxel as first-line therapy in women with MBC.

Methods: Eligible pts have Stage IV, HER2-negative MBC; ECOG PS 0-1; and adequate organ function. No prior chemotherapy is allowed (other than 1 regimen in the adjuvant setting). Tesetaxel was administered orally Q3 wks at a starting dose of 27 mg/m² with escalation to 35 mg/m² as tolerated. No premedication for potential hypersensitivity was used. RECIST response rate was the primary endpoint. A Simon min-max two-stage design was used with a target response rate of 30% in 25 pts.

Results: To date, 20 women have been enrolled and treated. The median age was 62 years (range, 45-78). Time from diagnosis was > 4 years in 5 pts and ≤ 4 years in 6; MBC was newly diagnosed in the remaining 9. Hormone receptor status was triple negative in 5 pts at diagnosis and 10 at the time of metastasis. The most common sites of metastases were lung (13 pts) and bone (9). Prior treatment included hormonal therapy in 13 pts, adjuvant chemotherapy in 16 (most commonly, ACT), and radiotherapy in 9. Of 11 pts currently evaluable for response, PR was achieved in 6 (55%), with confirmation of response in 4 and an ongoing PR in 1 of the 2 pts with an unconfirmed PR. SD was observed in 2 and disease progression in 3. Neutropenia was the most common adverse event, affecting 50% of pts; Grade 3-4 neutropenia occurred in 13.3%, G2 neuropathy occurred in 15%. Median time to progression & median survival were 18.5 & 28 months respectively.

Conclusions: The overall response rate achieved with weekly paclitaxel plus paraplatin is among the highest in metastatic breast cancer. However, the poor response rate seen in patients with p53 overexpression for response, PR was achieved in 6 (55%), with confirmation of response in 4 and an ongoing PR in 1 of the 2 pts with an unconfirmed PR. SD was observed in 2 and disease progression in 3. Neutropenia was the most common adverse event, affecting 50% of pts; Grade 3-4 neutropenia occurred in 13.3%, G2 neuropathy occurred in 15%. Median time to progression & median survival were 18.5 & 28 months respectively.

P5-19-12

Integration of Capecitabine Monotherapy with Capecitabine Combination Therapy in Metastatic Breast Cancer Patients: First Report on Safety and Efficacy of Single Agent Capecitabine Maintenance Study.

Xu B, Wang J, Yuan P, Ma F, Li Q, Zhang P, Fan Y, Li Q. Cancer Hospital Chinese Academy of Medical Sciences, Beijing, China

Background: Giving the high degree of efficacy and safety profile, Docetaxel/Paclitaxel in combination with Capecitabine are indicated as 1st line standard chemotherapy for anthracycline pretreated or resistant metastatic breast cancer (MBC) patients. Maintenance chemotherapy is chemotherapy administered to patients without progression after an initial chemotherapy, generally as 1st line treatment. Unfortunately, no clinical studies has prospectively evaluated the efficacy and safety of capecitabine single agent maintenance in MBC. Methods: Previously untreated patients with metastatic breast cancer (MBC) were randomly assigned to receive Capecitabine 1,000 mg/m² orally twice daily on day 1-14, Vinorbinb 25 mg/m² on day 1 and 8 (NX) in 21-day cycle or Capecitabine 1,000 mg/m² orally twice daily on day 1-14, Docetaxel 75 mg/m² on day 1 (TX), in 21-day cycle. The primary endpoint was to compare progression free survival (PFS), second endpoints were safety profiles (NCI-CTC 3.0), overall survival and response rate. During Initial study treatment, 21 days as one cycle and 6-8 cycles are required; Patients who were responding (complete or partial), or whose diseases were stable followed by Capecitabine: 1,000 mg/m² PO twice daily (day 1-14) 21 days as one cycle until progression or unacceptable toxicity. This was designed as a non-inferiority study. The efficacy analysis was based on the intent-to-treat population (all patients randomized and received at least one dose of test drug). Safety was assessed on the safety population who received at least one dose of study medication. Results: Ninety one women were enrolled in fourteen months. Fifty six patients were treated with TX and 35 with NX. The median PFS was 7 (NX) versus 9 months(NX) (P=0.1888). According to RECIST criteria, similar objective response rates (19(54.2%) on TX versus 28(50%) on NX, P= 0.158)
clinical benefit (CR+PR+SD) [29(82.9%) on TX versus 48(85.7%) on NX, P=0.216] were achieved. The main adverse events included grade 3-4 neutropenia (40.0% versus 30.4%; P=0.115), hand-foot syndrome (2.8% versus 12.5%; P=0.006), grade 2-3 gastrointestinal adverse events (37.2% versus 19.7%; P=0.037), myalgia and arthralgia (2.9% versus 5.4%; P=0.363), with NX and TX, respectively. 2.8% versus 12.5% were discontinued treatment for toxicity with NX and TX(P=0.092). Numbers of patients transferred into maintenance treatment with Cepacitabine were 22(70.9%) versus 36(64.3%) in NX and TX arm respectively. 13.6% versus 13.9%(P=0.303) of the patients were discontinued treatment for toxicity with NX and TX respectively. The main toxicity in maintenance phase was grade 2-3 hand-foot syndrome. The median cycles of maintenance treatment was 4 cycles, 4 patients received Cepacitabine maintenance treatment for more than 12 cycles. Discussion: There are few clinical study to compare the two regimens, especially with Cepacitabine maintenance treatment. Our preliminary study found that NX and TX regimen have similar efficacy but different toxicity. Both regimen can be used as front-line treatment of MBC.

P5-19-13
A Randomized Phase II Trial of First-Line Metastatic Breast Cancer (MBC) Patients: Sub-Set Analysis of Albumin-Bound Paclitaxel (ab-pac) Given Weekly at 150 mg/m².

Gradishar WJ, Krasnojon D, Cheporov S, Makhson AN, Manikhas GM, Clawson A, Bhar P. Northwestern University Feinberg School of Medicine, Chicago, IL; Leningrad Regional Oncology Center, Russian Federation; Yaroslavl Regional Clinical Oncology Hospital, Yaroslavl, Russian Federation; City Oncology Hospital, Russian Federation; St. Petersburg City Oncology Center, St. Petersburg, Russian Federation; Celsegne Corporation, Summit, NJ

Background: We previously reported the results of a phase II study evaluating the efficacy and safety of 3 different dosing regimens of ab-pac and docetaxel for the first-line treatment of MBC (Gradishar et al. J Clin Oncol. 2009;27:3611). Here we report outcomes for a subset of patients (pts) during treatment with ab-pac at 150 mg/m² weekly for the first 3 weeks of a 4-week schedule (qw 3/4). Methods: Patients (N = 300) with previously untreated MBC were randomized to 1 of 4 treatment arms: arm A, ab-pac at 300 mg/m² qw3; arm B, ab-pac at 100 mg/m² qw 3/4; arm C, ab-pac at 150 mg/m² qw 3/4; arm D, docetaxel at 100 mg/m² qw3. A step-down statistical approach was used for pairwise comparisons of treatment groups. The trial was powered for antitumor activity and safety. Results: Treatment arm C produced the longest overall survival (OS) (33.8 months) with an 11.6-month longer median OS vs arm B (22.2 months, HR 0.575; P=.008) and a 7.2-month longer median OS vs arm D (26.6 months, HR 0.688; P not statistically significant). Median OS in arm A was 27.7 months. These OS data were consistent with previously published overall response rates (ORR) and progression-free survival (PFS). Forty-seven percent of pts in arm C required dose reduction due to toxicity, including 27% due to neutropenia (np), 15% due to sensory neuropathy (SN), 3% due to allergy/immunology, 1% due to febrile np, and 1% due to ulceration of the skin. The median OS for the subset of pts requiring DRs in arm C was comparable to pts not dose reduced: 35.2 and 31.8, respectively. Pts who were dose reduced in arm C received a median of 2 additional cycles of treatment compared with those without DRs: 10 (range 2 – 27) vs 8 (range 1 – 27). Investigator assessed ORR and PFS were numerically higher in pts dose reduced vs those not reduced. Baseline characteristics were similar between pts requiring DRs vs not.

P5-19-14
Platinum-Based Chemotherapy in Triple-Negative Breast Cancer. Villarreal-Garza C, Bouganim N, Khalaf D, Clemons M, Kassam F, Enright K, Verma S, Myers J, Dent R. Sunnybrook Odette Cancer Centre, Toronto, ON, Canada; The Ottawa Hospital Cancer Centre, Ottawa, ON, Canada; Princess Margaret Hospital, Toronto, ON; Credit Valley Hospital, Mississauga, ON, Canada

Background: There is increasing evidence that DNA repair defects characteristic of BRCA1-related cancers and triple negative breast cancer (TNBC) confer sensitivity to certain chemotherapeutic agents, such as platinum. However, prospective and retrospective studies comparing the efficacy of these agents versus conventional treatment in TNBC are lacking. The aim of this study was to evaluate the efficacy of platinum-based chemotherapy (PBC) in metastatic TNBC in terms of median duration of treatment and overall-survival (OS) and compare it to patients treated with conventional chemotherapy. Methods: We performed a retrospective chart review of patients with metastatic TNBC who received PBC from January 2007 to June 2010 treated at the Sunnybrook Odette Cancer Center and the Ottawa Hospital Cancer Centre. This cohort was compared to a control group that included metastatic TNBC treated with conventional agents that included anthracyclines, taxanes, capecitabine, and vinorelbine. Results: A total of 166 metastatic TNBC patients were analyzed: 60 treated with PBC and 106 managed with conventional treatment. Median age at diagnosis was 48 years and distant disease-free interval was 26 months (m) for both groups. Patients on both groups had multiple sites of metastases at diagnosis of recurrence than a single site of metastasis (69% for both groups). Of the 60 patients treated with PBC, 90% received a combination regimen, most commonly weekly cisplatin plus gemcitabine in 37% of patients and cisplatin plus vinorelbine in 17% of patients. The median number of cycles delivered was 4 (1-24). 33% received the PBC as first-line treatment, 38% as second-line, 18% as third-line, 7% as fourth line, and 3% as fifth-line. Only 8 patients (5%) discontinued PBC secondary to toxicity. The median time on treatment in first, second and third-line therapy was longer for the PBC group compared to the conventional

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<th>Baseline Characteristics</th>
<th>Ab-pac 150 mg/m² qw 3/4</th>
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Clinical Outcome
ORR, % (95% CI) 80 (67-93) 69 (55-84)
PFS in months, median 14.8 12.3
OS in months, median 35.2 31.8
Pts on secondary therapies, n (%) 31 (89) 26 (67)

Safety
Neutropenia, n (%) Grade 3 13 (34) 8 (24)
Neutropenia, n (%) Grade 4 1 (3) 1 (3)

group (5 vs. 2 m, p=0.108; 5 vs. 2 m, p=0.01; and 4 vs. 1 m, p=0.026). Patients treated with PBC had a longer OS compared to those managed conventionally (16 vs. 10 m, p=0.039).

Conclusions:
PBC appears to improve clinical outcomes in patients with metastatic TNBC compared to those treated with conventional chemotherapy regimens. Although this is a retrospective study with its obvious limitations, it adds to the growing body of literature, suggesting the benefit of PBC in TNBC. Prospective trials are needed to confirm its benefit in order to integrate it as part of the routine management of these patients.

P5-20-01
Patient Valuation of Reduced Risk of Side Effects during Treatment for Metastatic Breast Cancer.
Lalla D, McLaughlin T, Brammer M, Bramley T, Bare A, Carlton R. Genentech Inc., S San Francisco, CA; Xcenda, Palm Harbor, FL

Background: Chemotherapy treatment for metastatic breast cancer (MBC) has shown significant benefits in survival for breast cancer patients. However, chemotherapy is associated with several side effects that have a significant impact on patients’ quality of life. The objective of this analysis was to quantify the value patients with MBC place on reduced risk of treatment side effects.

Methods: A willingness to pay (WTP) survey was developed to assess metastatic breast cancer patients’ willingness to pay for a reduction in the risk of breast cancer treatment side effects. The survey assessed patients’ WTP for a 25%, 50% and 100% reduction in the risk of all side effects. Patients were also asked to select the side effect they would pay the most to avoid. Additionally, the survey collected demographic information such as treatment regimen, age, race/ethnicity, region, employment status, and insurance type.

Results: The survey was completed by 202 metastatic breast cancer patients. Most survey respondents were white (94%), married (62%), and over the age of 51 (78%). Most patients were covered by private insurance (67%) or Medicare (24%). Of the 58% of respondents who remembered paying out of pocket for their last treatment, the average out of pocket payment was $459. Survey respondents were willing to pay an extra $1,886, $3,837 and $7,794 for a 25%, 50% and 100% reduction in the risk of all side effects, respectively. Hair loss (28%), pain (17%) and nausea (15%) were selected most often as the side effect they would pay the most to avoid.

Discussion: Chemotherapy for MBC is associated with several adverse events that patients would like to avoid or reduce such as hair loss, pain, nausea and neutropenia. This analysis demonstrates patients with MBC place a significant value on reducing the risk of side effects and are willing to pay 4.2 times for a treatment devoid of side effects as compared to a treatment with a 25% reduction in the risk of treatment side effects.

P5-20-02
N-Terminal Pro-Brain B-Type Natriuretic Peptide (nt-pro-BNP) before and during Treatment with Trastuzumab Allows Early Detection of Cardiotoxicity in Breast Cancer Patients.
Blancois I, Carrillo J, Legérén M, Delgado M, Jurado JM, Zarcos I, Villaescusa A, Gómez FJ, Moreno E, Garcia-Puche JL. Hospital Clínico San Cecilio, Granada, Spain; University of Granada, Granada, Spain

Background: The treatment with Trastuzumab (T) is associated with a certain degree of cardiotoxicity. This study sought to evaluate the level of nt-pro-BNP as a possible marker of cardiotoxicity so accurate as the value of left ventricular ejection fraction (LVEF)

Patients and methods: Forty patients with breast cancer treated with T were prospectively measured LVFE with echocardiography and nt-pro-BNP level before and every 3 months during T treatment. Median age: 50years(27-70). Received T as adjuvant treatment: 82.5% of the patients and 17.5% for metastatic disease. Patients with previous chemotherapy: 13(32.5%),neoadjuvant, 23(57.5%)adjuvant and 7(17.5%) for metastatic disease. Patients with previous treatment with anthracyclines: 12(30%)neoadjuvant, 21(52.5%)adjuvant and 2(5%) for metastatic disease(one patient had received anthracyclines too during adjuvant treatment).None received anthracyclines and T concomitant. Chi-square analyzed the correlation of high pathological level of nt-pro-BNP (values over the normal range,adjusted by the patient age) and a significative decrease of LVEF (more than 10% when LVEF >50% or more than 5% when LVEF<50%).

Results: A decrease in LVEF was observed in 6 patients(15%) and pathological high levels of nt-pro-BNP in 7(17.5%).Two patients(5%) presented cardiac insufficiency with clinical symptoms, in both nt-pro-BNP was higher than 600pg/ml and LVEF lower than 40%. In the other patients the decreases of LVEF (never >40%) or high levels of nt-proBNP (never >600pg/ml) were not related to any clinical symptoms. Chi-square analysis showed a correlation between pathological high levels of nt-pro-BNP and significative decrease of LVEF (p=0.001). The estimated risk of a false negative nt-pro-BNP result (nt-pro-BNP normal value and significative LVEF decrease) was 2.735% (95% confidence interval: 0.878 to 8.522 %) and the estimated risk of a false positive nt-pro-BNP result (nt-pro-BNP pathological value and no significative LVEF decrease) was 0.132%(95% confidence interval: 0.39 to 0.448%)

Conclusion:The level of nt-pro-BNP is a possible marker of cardiotoxicity so accurate as the value of LVEF in patients with breast cancer treated with T.

P5-20-03
Kaderer NM, Cuklakova E, Poniewierski MS, Crawford J, Dale D, Lyman GH. Duke University; Durham, NC; University of Washington, Seattle, WA

Background: Neutropenic complications including severe and febrile neutropenia (FN) represent major dose-limiting toxicities of cancer chemotherapy. A general risk model for neutropenic complications across major solid tumors has been developed and validated (Lyman et al. Cancer 2011). Current guidelines recommend consideration of primary prophylaxis with a colony-stimulating factor (CSF) in patients at >20% risk of FN. The decision for primary CSF prophylaxis in patients on intermediate risk chemotherapy (10-20%) is based on physician assessment of individual patient risk factors for FN. This study assesses the ability of this general FN risk model to identify ESBC patients on intermediate risk chemotherapy who are at a personal high risk for developing a neutropenic complication.

Methods: A prospective cohort study accrued 4458 consenting patients starting a new chemotherapy regimen at 115 randomly selected community oncology practices throughout the United States from 2002-2006. The risk of severe or febrile neutropenia (SNFN) in cycle 1 and across 4 cycles was estimated [±95% CI] utilizing logistic regression analysis and adjusting for key clinical factors including among others: age, prior chemotherapy, abnormal hepatic or renal function, low pretreatment white blood count, immunosuppressive medications, CSF prophylaxis, and planned relative dose intensity.
as well as major chemotherapeutic agents. The cumulative risk of FN across 4 cycles was also estimated by the product limit method of Kaplan and Meier.

Results: Among 1224 patients with ESBC, 822 received intermediate risk chemotherapy based on National Comprehensive Cancer Network guidelines. Among these patients, cycle 1 SNFN occurred in 37%, at least one episode of FN over 4 cycles of chemotherapy in 17%, with 15% receiving primary CSF prophylaxis. The predicted risk of cycle 1 SNFN ranged from 1%-79%, with mean (median) risk of 33.8% (39.0%). Model performance was good with a c-statistic of 0.73 [0.69-0.76]. Based on this general FN risk model, cycle 1 SNFN occurred in 47% of predicted high risk ESBC patients [42 - 52%] compared to 13% [8-17%] of low risk patients. One or more FN events over 4 cycles occurred in 20% [17-24%] of predicted high risk versus 10% [6-14%] in low risk patients. The cumulative risk of FN by Kaplan-Meier estimation was 23% in high risk and 10% in low risk patients. Model sensitivity and specificity for FN were 83% and 33%, respectively. The majority of SNFN (76%) and FN (58%) events among high risk patients occurred in cycle 1. 50% of high risk patients who did not receive primary CSF prophylaxis went on to receive CSF during subsequent cycles.

Conclusions: Based on good test performance characteristics, this clinical FN prediction model also identifies ESBC patients receiving intermediate risk chemotherapy at high personal risk for FN (FN >20%) over the first 4 cycles of chemotherapy. Half of predicted high risk patients without primary CSF prophylaxis will be given CSF in subsequent cycles after the occurrence of a neutropenic complication. This also confirms previous clinical trial findings that the majority of febrile neutropenic events occur in the first cycle.

**P5-20-04**


Younis T, Rayson D, Thompson K. Dalhousie University at the Queen Elizabeth II Health Sciences Centre, Halifax, NS, Canada

**Background:** Adjuvant chemotherapeutic regimens incorporating docetaxel, including TC (taxotere 75 mg/m²; cyclophosphamide 600 mg/m² q 3 weekly for 4 cycles) and FEC-D (5-flououracil 500 mg/m²; epirubicin 100 mg/m²; cyclophosphamide 500 mg/m² q 3 weekly for 3 cycles followed by docetaxel 100 mg/m² q 3 weekly for 3 cycles), appear to be associated with rates of febrile neutropenia (FN) in routine clinical practice that are higher than those reported in the pivotal clinical trials (7-33% vs. 11% & 18-35% vs. 5%, respectively). Although primary prophylaxis with G-CSF (granulocyte colony-stimulating factor) is indicated for chemotherapeutic regimens associated with rates of FN >20%, the variable FN rates reported with TC and FEC-D outside of clinical trials have precluded definitive recommendations for G-CSF primary prophylaxis in most jurisdictions. A systematic review and meta-analysis was therefore conducted to assess; i) FN rates associated with TC and FEC-D without and with G-CSF primary prophylaxis outside of clinical trial settings, and ii) the potential impact of G-CSF prophylaxis on FN prevention.

**Methods:** A PubMed search was conducted and major conference abstracts were reviewed up to June 15th 2011 to identify all English language reports of FN rates associated with adjuvant TC or FEC-D outside of clinical trial settings. FN rates with and/or without G-CSF prophylaxis were abstracted, and LOS (length of stay in hospital) and mortality following FN were noted. Summary incidences and odds ratios (OR) with 95% confidence intervals (95%CI) were calculated using random- and fixed-effects models.

**Results:** A total of 902 patients treated with TC (average age 55 years, range 27-84, 19% ≥ 65 years) and 1342 treated with FEC-D (average age 52 years, range 24-78, 9% ≥ 65 years) from 13 and 9 relevant studies respectively, were included. Overall FN rates of 17% (range: 7-33%) and 24% (range: 18-35%) were reported for TC and FEC-D, with an average primary G-CSF utilization rate of 47% and 30%, respectively. For TC, the pooled random-effects meta-analysis estimates of FN rates were 7% (95%CI: 5-10%) with primary G-CSF and 29% (95%CI: 24-35%) without G-CSF (OR=0.17, 95%CI: 0.09-0.33). For FEC-D, the FN rates were 9% (95%CI: 4-19%) with and 31% (95%CI: 27-35%) without primary prophylaxis (OR=0.22, 95%CI: 0.09-0.56). For FEC-D, 50% of the FN events occurred during the 1st D cycle and 65% overall occurred during D treatments. Older age (≥ 65 years) did not appear to correlate with higher FN rates. Breakthrough FN occurred in 5% across both regimens despite G-CSF secondary prophylaxis. FN was associated with 3.7 and 4.0 LOS days and 0% and 2% mortality during TC and FEC-D, respectively.

**Conclusions:** TC and FEC-D with or without G-CSF are associated with unacceptably high FN rates in routine clinical practice. Primary prophylaxis with G-CSF should be considered for adjuvant TC chemotherapy, and for the D-component of FEC-D regimen, irrespective of patient age.

**P5-20-05**

Peripheral Blood Transcriptomics and Doxorubicin Cardiotoxicity.

Todorova VK, Beggs ML, Dhakal IB, Henningis LJ, Makhoul I, Klimberg VS. University of Arkansas for Medical Sciences, Little Rock, AR

**Background:** Doxorubicin (DOX) cardiotoxicity is dose-dependent and unpredictable. Currently, the clinical methods used for detection of pre-symptomatic DOX-induced cardiotoxicity show low diagnostic sensitivity and identify the existing cardiomyopathy rather than prevent it. It has been reported that DOX cardiomyopathy can occur at low doses, suggesting the presence of increased phenotypic sensitivity by some individuals. Studies have found specific gene expression signatures of circulating blood cells in response to physiological and pathological changes, suggesting that they can be used as biosensors for diagnostic purposes. This study aimed to characterize the peripheral blood mononuclear cells (PBMC) gene expression profile associated with DOX-induced cardiotoxicity with an ultimate goal to identify biomarkers for early prediction of DOX cardiotoxicity.

**Materials and Methods:** Twenty Sprague Dawley rats were randomized into 2 groups: DOX-treated (n=12) and controls (n=8). DOX-treated rats received a single intraperitoneal dose of 12 mg/kg DOX (similar to 65mg/m² for humans) and the controls were injected with saline. All rats were sacrificed 48 hours after DOX administration and blood, and samples from the heart left ventricle (LV) were collected. Histopathological alterations were evaluated microscopically. RNAs was isolated from cardiac LV and PBMCs. Cardiac and PBMC genome-wide expression profiling were performed using illumina Rat Ref-12 BeadChip microarrays. For statistical analysis the raw data were log, transformed and median normalized. The differential analysis per gene was performed using a 2-sample (control vs. DOX-treated) Student’s t-test. Statistical significance was set at false discovery rate (FDR)<0.05. Differentially expressed genes were mapped to known metabolic/signaling pathways using MetaCore GENEGO software.

**Results:** A total of 1406 transcripts were differentially expressed in the hearts (247 upregulated and 1159 downregulated) and 1526 in PBMCs (271 upregulated and 1255 downregulated) between DOX-treated rats and control rats. Of these 1237 genes (92%) were similarly differentially regulated (179 upregulated and 1058 downregulated) both in the hearts and PBMCs. Fifty metabolic/signaling pathways
were significantly affected by DOX-treatment. The top ten of these include oxidative phosphorylation; cell proliferation and differentiation; TGF, WNT and cytoskeletal remodeling; insulin signaling; cell adhesion; regulation of amino acid metabolism, cytoskeleton remodeling, PI3K/Akt signaling and CCR5 signaling in macrophages and T lymphocytes.

Discussion: The results from this study showed that PBMC gene expression profile reflected a similar pattern in the hearts of rats treated with DOX and provide a novel and important information about the feasibility of the PBMC as a surrogate marker for DOX cardiotoxicity. The data obtained lay a foundation for further clinical studies to identify the cardiotoxicity risk, predict DOX-treatment response and ultimately to allow the anti-cancer therapy to be tailored to individual patients.

P5-20-06
Survival in Women with Breast Cancer Who Used or Did Not Use Scalp Cooling in the Neoadjuvant/Adjuvant Setting.
Lemieux J, Perron L, Provencher L, Brisson J, Amireault C, Blanchette C, Maunsell E. Centre de Recherche FRSQ du CHA Universitaire de Quebec, Quebec City; QC, Canada; Hôpital du Saint-Sacrement, Quebec City; QC, Canada; Université Laval, Quebec City; QC, Canada; Institut Nationale de Santé Publique, Quebec City; QC, Canada; Université de Montréal, Montreal, QC, Canada

Background: Scalp cooling can prevent chemotherapy-induced alopecia. Success varies according to the type of chemotherapy. A controversy exists regarding the use of scalp cooling because of the lack of safety data. No data are available regarding the impact on survival.

Purpose: To compare overall survival in women who used or did not use scalp cooling in the neoadjuvant/adjuvant setting.

Method: The survival of women treated in a specialized breast cancer centre (the Centre des Maladies du Sein Deschênes-Fabia) in Quebec City who all used scalp cooling was compared to that of a population-based random sample of women treated in other regions of the province of Quebec (Canada) where scalp cooling is not available.

Cox proportional hazard models were used.

Results: Overall, survival was comparable (and possibly better although not at a conventionally statistically significant level: HR = 0.80, 95% CI = 0.63–1.01, p=0.06) among the 553 women who used scalp cooling compared to the 817 who did not. An interaction was found between scalp cooling and treatment in the adjuvant vs. neoadjuvant setting (p-interaction=0.015). In the adjuvant setting (n=485 scalp cooling and 740 no scalp cooling), the crude HR (in favour of scalp cooling) was 0.66 (95% CI: 0.50-0.87, p=0.003). In the neoadjuvant setting (n=68 scalp cooling and 77 no scalp cooling), the HR was 1.40 (95% CI: 0.84-2.33, p=0.2). No interaction was found with stage.

Conclusion: This is the first study to compare survival of women who used scalp cooling to that of women who did not. Scalp cooling to prevent chemotherapy-induced alopecia had no negative effect on survival in women with breast cancer who used it.

P5-20-07

Background: US Oncology Trial 9735 (Jones S, et al. JCO. 2006;24:5381-5387) established the docetaxel plus cyclophosphamide (TC) regimen as an effective adjuvant therapy for early stage breast cancer (ESBC). This trial did not specifically evaluate the incidence of febrile neutropenia (FN) as a study endpoint, but rates of 4%–8% were reported. Prophylactic granulocyte colony-stimulating factor (G-CSF) support was not allowed; reactive G-CSF support overall was not reported. Subsequent reports in the community setting have indicated FN rates of 25%–50% without G-CSF support and 0%–6.3% with G-CSF support (Table 1). To better determine the incidence of FN among ESBC patients treated with TC, we performed a retrospective clinical data review from the electronic medical record (EMR) database of Georgia Cancer Specialists, a large community oncology practice.

Methods: EMR data were captured between January 2006 and March 2010. Eligibility included women ≥ 18 years old with ESBC (stage I–IIIa) who completed ≥ 1 cycle of TC. The study time period was from the first dose of chemotherapy (CTX) to 6 weeks after the last dose of CTX, death, or loss to follow-up. The primary endpoint was the incidence of FN. Other endpoints included the incidence of severe (grade 3/4) neutropenia, neutropenia-related hospitalizations, G-CSF use, relative dose intensity (RDI), and dose delays and reductions.

Results: Data from 662 patients were included in the analysis. Median age was 55 (range: 25–81) years. 40% of patients were white. The median number of CTX cycles received was 4 (range: 1–6). Most patients (91%) received G-CSF support; 73% as primary prophylaxis. See Table 2 for additional results.

Conclusions: This is the largest retrospective, community-based study to evaluate the incidence of FN in ESBC patients treated with TC. The observed FN rate of 5% (with 91% of patients receiving G-CSF) is consistent with other published reports using TC (Table 1). Our results suggest that TC is a taxane regimen with clinically significant myelosuppression (similar to other commonly used regimens in ESBC, such as TAC [NCCN Guidelines v2.2011]) and that the use of G-CSF support should always be considered.

Table 1.

<table>
<thead>
<tr>
<th>Study</th>
<th>FN Rate (N)</th>
<th>Metyrosine T</th>
<th>Reference</th>
<th>NCCN Guidelines</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soong D, et al. JCO 2009;27:e101-2.</td>
<td>31 (57)</td>
<td>22 (3)</td>
<td>31 (57)</td>
<td>22 (3)</td>
</tr>
<tr>
<td>Chai A, et al. Support Care Cancer 2011;19:497-504.</td>
<td>37 (2)</td>
<td>50%</td>
<td>39 (2)</td>
<td>50%</td>
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</tbody>
</table>

Table 2.

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>N = 662</th>
<th>USP</th>
<th>CTC</th>
<th>NCCN Guidelines</th>
</tr>
</thead>
<tbody>
<tr>
<td>FN overall/cycle 1</td>
<td>31 (57)</td>
<td>22 (3)</td>
<td>31 (57)</td>
<td>22 (3)</td>
</tr>
<tr>
<td>Grade 3/4 neutropenia overall/cycle 1</td>
<td>282 (43)</td>
<td>182 (25)</td>
<td>282 (43)</td>
<td>182 (25)</td>
</tr>
<tr>
<td>Neutropenia-related hospitalizations</td>
<td>25 (4)</td>
<td>25 (4)</td>
<td>25 (4)</td>
<td>25 (4)</td>
</tr>
</tbody>
</table>

G-CSF use

<table>
<thead>
<tr>
<th>Category</th>
<th>N</th>
<th>USP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>604 (91)</td>
<td>604 (91)</td>
</tr>
<tr>
<td>Primary prophylaxis</td>
<td>486 (75)</td>
<td>486 (75)</td>
</tr>
<tr>
<td>Secondary prophylaxis</td>
<td>57 (9)</td>
<td>57 (9)</td>
</tr>
<tr>
<td>Treatment/salvage</td>
<td>61 (9)</td>
<td>61 (9)</td>
</tr>
<tr>
<td>Completed ≤4 cycles of CTX</td>
<td>556/632* (88)</td>
<td>556/632* (88)</td>
</tr>
<tr>
<td>RD ≤ 20%</td>
<td>522/998* (89)</td>
<td>522/998* (89)</td>
</tr>
<tr>
<td>Dose delays (interval &gt; 28 days)</td>
<td>24/352* (4)</td>
<td>24/352* (4)</td>
</tr>
<tr>
<td>Dose reductions (&lt; 20%)</td>
<td>28/632* (4)</td>
<td>28/632* (4)</td>
</tr>
</tbody>
</table>

* 30 patients who had modifications to the TC regimen after the 1st cycle were excluded; 53 patients who did not complete 4 cycles of CTX by the study cut-off date were excluded. RDI was calculated over cycles 1–4.
P5-20-08
Multicenter Results of Scalp Cooling To Prevent Chemotherapy-Induced Alopecia in 1500 Breast Cancer Patients.
van den Hurk C, Peerbooms M, Komen M, Nortier H, Breed W. Comprehensive Cancer Centre South (IKZ), Eindhoven, Netherlands; Medical Centre Alkmaar, Alkmaar, Netherlands; Leiden University Medical Centre, Leiden, Netherlands

Background
Chemotherapy-induced alopecia (CIA) is a frequent occurring side effect of cancer treatment that has high psychological impact on many patients and their relatives. CIA may be prevented by scalp cooling.

Methods
Breast cancer patients who received scalp cooling could participate in this registration study from 2006 and onwards. Nurses and patients completed questionnaires. Scalp cooling was performed using the Paxman PSC1 or PSC2 system and was considered satisfying when patients did not wear a wig or head cover. Logistic regression analyses will be used to examine determinants of the scalp cooling result, including age, type / length / thickness / chemical manipulation (dyeing, waving, colouring) of hair, type / dose of chemotherapy, cytostatic infusion time, post-infusion cooling time, dampering hair or use of conditioner before scalp cooling and previous treatment with chemotherapy.

Results
The use of scalp cooling increased from 4 hospitals in 2005 to 60 out of 100 Dutch hospitals in 2011. The registration comprised about 1500 scalp cooled breast cancer patients of whom 79% were treated in the adjuvant setting. Overall, patients' satisfaction with the result of scalp cooling was 50%, but varied for different chemotherapy schemes and dosages from 8% in docetaxel 75 mg/m², doxorubicin 50 mg/m², cyclophosphamide 500 mg/m² (TAC) to 81% in paclitaxel 70-90 mg/m². The results of the regression analyses will be presented at the conference.

Discussion
Scalp cooling is effective in most commonly used chemotherapy regimens for breast cancer patients. Therefore, scalp cooling should be offered more often. Efficacy depends on the type and dose of chemotherapy. Other factors influencing the result have never been studied in multivariate analyses before. Scalp cooling is ineffective in patients treated with a combination of a taxane and an anthracyclin. The frequent use of scalp cooling in the adjuvant setting indicates minimal fear for an increased incidence of scalp skin metastases after scalp cooling in breast cancer patients. Multicenter registration of results improves information for medical professionals and patients and will lead to more frequent use of scalp cooling and improvement of the method.

P5-21-02
The Susan G. Komen for the Cure® Tissue Bank at the IU Simon Cancer Center: The Source for Normal Breast Tissue and Biospecimens.
Clare SE, Mathieson T, Henry JE, Zhang H, Way ES, Ridley KE, Badve S, Herbert B-S, Rufenbarger CA, Storniolo AMV. Indiana University School of Medicine, Indianapolis, IN

Background:
Our efforts to prevent and treat breast cancer are significantly impeded by a lack of knowledge of the biology and developmental genetics of the normal mammary gland. This ignorance has been the consequence of the lack of access to richly annotated, high quality normal breast specimens. The Susan G. Komen for the Cure® Tissue Bank at the IU Simon Cancer Center (KTB) was established expressly to remedy this deficiency. The KTB is a repository of specimens from volunteer donors with no clinical evidence of breast malignancy. The Bank’s mission is to make available specimens that will enable an understanding of the developmental biology of the normal breast, to provide insight into breast oncogenesis, and to provide a normal control for breast cancer research. The purpose of this presentation is to increase the awareness of this unique and rich research resource and to actively solicit the use of its specimens.

Methods:
The KTB has been prospectively banking fresh frozen breast tissue since mid-2006. Coincident with the tissue donation that traditional immunocompromised mouse models are not generally permissive for growth. We sought to circumvent some of these limitations by transplanting and growing human mammary tumors in the mammary fat pad of SCID/Beige immunocompromised mice in the absence of exogenous human fibroblasts.

Aims and Methods
To establish a set of stable human breast cancer xenografts for preclinical studies. Human breast cancer biopsies were received, minced into small fragments and then transplanted directly into “cleared” fat pads of recipient SCID/Beige immunocompromised mice. Transplanted fat pads were checked weekly. After initial tumor was palpated and harvested, tumor fragments were transplanted into new SCID/Beige hosts for subsequent transplant generations. Serial immunohistochemical evaluations were performed to confirm human origin and biomarker status. Analytical flow cytometry for evaluating expression of proposed “cancer stem cell” markers, and gene and protein expression analysis were carried out on all stable lines.

Results and Conclusions
Xenograft lines were established directly from breast cancer patient samples, without intervening culture in vitro, using the epithelium-free mammary fat pad as the transplantation site. Of the conditions tested, xenograft take rate was highest in the presence of a low-dose estradiol pellet without exogenous human fibroblasts. Thirty six stably transplantable xenograft lines representing 27 patients were established, using pre-treatment, mid-treatment, and/or post-treatment samples. Most patients yielding xenografts were “triple-negative” (ER-PR-HER2-) (n=21), we were able to establish lines from three ER-PR-HER2+ patients, one ER+PR-HER2-, one ER+PR-HER2- and one “triple-positive” (ER+PR+HER2+) patients. Serially passaged xenografts show phenotypic consistency with the tumor of origin at the histopathology level, and remarkable stability across multiple transplant generations at both the genomic, transcriptomic, and proteomic levels. Of 27 lines evaluated fully, thirteen xenografts showed metastasis to the mouse lung. These models thus serve as a renewable, quality-controlled tissue resource, and should prove useful for preclinical evaluation of experimental therapeutics.

P5-21-01
Zhang X, Dobrolecki LE, Lai Q, Landis MD, Wong H, Tsimelzon A, Claerhout S, Contreras A, Gutierrez C, Huang J, Wu M-F, Pavlik AC, Froehlich AM, Hilsenbeck SG, Mills GB, Wiechmann L, Petrovic I, Rimawi MF, Schiff R, Chang JC, Lewis MT. Baylor College of Medicine, Houston, TX; The University of Texas MD Anderson Cancer Center, Houston, TX

Introduction
Translational breast cancer research is hampered severely by difficulties in obtaining and studying primary human breast tissue, and by the lack of in vivo preclinical models that accurately reflect patient tumor biology. These limitations are due, in part, to the fact...
two tubes of blood are obtained, which are processed for lymphocyte DNA, serum and plasma. These specimens are richly annotated with detailed information regarding the donors’ reproductive history, medical history, family history, and medications. Standard Operating Procedures have been constructed so as to control, limit and identify potential sources of bias. All of this information is recorded in an Oracle-based, searchable database.

Results: As of June 2011, the KTB and its predecessor bank, Mary Ellen’s Bank, have available fresh frozen breast tissue (10 gauge cores) from 1469 donors; formalin-fixed, paraffin-embedded tissue from 1055; DNA from 7507; serum from 2382; and plasma from 3771 donors. The KTB has also established 28 epithelial and 33 stromal cell lines from the cores; 4 of the epithelial cell lines have been immortalized using hTERT. Donors range in age from 18-86 years of age. 9% of donors to the KTB describe themselves as Hispanic/Latino. 5.2% of donors are Black or African-American. Using the Gail Risk Model, there is a bimodal distribution of life-time breast cancer risk among the donors: the largest peak is at 10% and a smaller one at 18%.

Conclusions: The KTB is a unique and invaluable research resource which is now open for business and accessible to researchers across the globe. We encourage researchers to avail themselves of this unique tissue resource and to also acquaint themselves with other sources of healthy breast tissue, i.e., the Love/Avon Army of Women [http://www.armyofwomen.org/].

P5-21-03
The Breast Cancer Campaign Tissue Bank.
Jones L, Chelada C, Ellis I, Ekbote U, Green A, Hanby A, Jordan L, Purdie C, Quinlan P, Speirs V. Barts Cancer Institute, London, United Kingdom; University of Nottingham, Nottingham, United Kingdom; University of Leeds, Leeds, United Kingdom; University of Dundee, Dundee, United Kingdom

The breast cancer research community has recognised that access to a source of carefully collected well-annotated human breast tissue is essential for translational research. Research institutions often face barriers in gaining access to this resource as collections typically have restrictive access policies or an over burdensome application process. This was formally recognised by around 50 prominent breast cancer researchers through a Gap Analysis conducted in London, UK in 2006. As a direct result of this report, 4 leading UK centres (Barts Cancer Institute, the Universities of Dundee, Leeds and Nottingham) with multi disciplinary expertise in pathology, basic science, bioinformatics and computer science have collaborated with a leading breast cancer charity to form the Breast Cancer Campaign Tissue Bank (BCCTB; http://www.breastcancercampaigntissuebank.org). BCCTB is a unique resource of biological materials and supportive clinical data, efficiently and ethically collected from patients with breast cancer, to provide researchers with high quality, relevant materials, helping to raise the standard of breast cancer research and facilitating the co-ordinated translation of scientific findings into the clinical setting. A wide range of biological materials are banked, including fresh frozen tumour and surrounding tissue, isolated purified cell populations (which can be provided for culture or DNA/RNA/protein extraction), whole blood and serum samples, as well as formalin-fixed paraffin-embedded material. Specialised collections are also available through the Bank on a collaborative basis. BCCTB has a centralised IT system allowing efficient tracking of samples and recording of raw data from studies, and providing a user-friendly web-based search portal to view material available. A purpose-built Bioinformatics platform allows mining breast cancer literature data from multiple sources and integrating different types of -omics and clinical data alongside publically relevant annotations from a growing number of biological resources such as NCBI, Ensembl, UniProt and Reactome. This platform is also fully interoperable with the International Cancer Genome Consortium (ICGC) and can be automatically cross-queried from the ICGC data portal which allows direct cross-comparison of experimental findings generated from the ICGC breast cancer projects with literature-derived information stored in our portal. Together this results in the highly efficient and co-ordinated use of samples, reducing duplication of effort and facilitating data mining and analysis. As science is constantly evolving we have an inbuilt R&D program, including cell immortalisation, investigating improved sample storage and collection methods and on-going IT development, all of which will ensure the bank remains cutting-edge. Tissue is released following review by a Tissue Access Committee comprising clinical and non-clinical breast cancer researchers and patient advocates. Direct interaction with end users ensures the materials and data supplied meets the researcher needs. Currently BCCTB is accepting applications from UK based researchers with projects funded by Breast Cancer Campaign. It will launch to the wider breast cancer community in the next 18 months.

P5-21-04
Patient Attitudes towards Undergoing Additional Breast Biopsy for Research Purposes.
Naim FM, Ballinger R, McLauchlan R, Hadjiminas DJ, Hogben K, Palmieri C, Cleator SJ. Royal Free Hospital, Royal Free Hampstead NHS Trust, London, United Kingdom; Brighton and Sussex Medical School, Brighton, United Kingdom; Charing Cross Hospital, Imperial NHS Healthcare Trust, London, United Kingdom

Background: Acquisition of additional breast tissue for research has become integral to breast oncology research but no data exists regarding patient attitudes to additional, non-diagnostic biopsies. Baseline research biopsies may be obtained as part of the diagnostic process as extra passage(s) of the needle through a ‘generic consent’ process, or as an additional biopsy event after carcinoma diagnosis has been confirmed and eligibility for a specific study has been established. This study sought to explore patient perspectives to such biopsies. Patients and Methods: Patients attending breast clinics were recruited to complete a questionnaire prior to their consultation (and any interaction with researchers or clinicians seeking consent for research biopsies) which examined willingness to undergo an extra fine needle aspirate (FNA) and/or core needle biopsy (CNB) for research either at time of the diagnostic biopsy (B1) or after diagnosis (B2). Descriptions of FNA and CNB procedures were supplied to those with no prior experience. Patient perspectives towards donating surplus tissue left over from a diagnostic procedure and/or surgery for storage for future research was also explored. Results: 100 patients were recruited, 42% with a prior history of breast carcinoma, 23% with family history of breast carcinoma and 65% 42% with previous experience of CNB/ FNA respectively for either benign or malignant disease. Willingness to undergo additional biopsy was greater for the B1 than the B2 time point, but equivalent for CNB and FNA (willingness CNB B1 50% vs B2 26%, willingness FNA B1 50% vs B2 29%). A statistically significant increase in willingness to undergo CNB and FNA at B1 and/or B2 was seen in association with prior diagnosis of breast cancer, family history of breast cancer, previous visit to breast clinic and prior experience of breast biopsy. Reasons for willingness or unwillingness were recorded. 83% of patients expressed a willingness to allow surplus tissue to be stored in a biobank for future research.

www.aacrjournals.org Cancer Res; 71(24 Suppl.) December 15, 2011 595s
**Conclusions:** When asked by questionnaire prior to clinic consultation, additional research biopsy was much more acceptable if undertaken at time of initial diagnostic biopsy rather than at a later time point. Acceptability was equivalent for CNB and FNA. Previous experience of biopsy, previous attendance to breast clinic, a prior history of breast cancer or family history of breast cancer increased acceptability suggesting that willingness is increased with knowledge of the biopsy procedure.

**P5-22-01**

Feasibility and Patient Safety of Serial Biopsies (bx) in Metastatic HER2-Positive Breast Cancer (BC) To Evaluate Alterations in Molecular Biomarkers (BM): Preliminary Results of SHERSig (Study of HER2 Signature in Metastatic Breast Cancer) a Prospective Phase II Study.

Chan A, Chan S, Price D, Bergh J, Lluch A, Redfern A, Chirgwin J, Lidbrink E, Dhadda A, López-Vega J, Lindman H, Beith J, Baron-Hay S, Kiernmaier A, Herbst F, Ellis I. Mount Medical Centre, Perth, Australia; Nottingham City Hospital, Nottingham, United Kingdom; Karolinska Institutet and University Hospital, Stockholm, Sweden; Hospital Clinico Universitario de Valencia, Valencia, Spain; Royal Perth Hospital, Perth, Australia; Eastern Health Melbourne, Australia; Scarborough Hospital, Scarborough, United Kingdom; Hospital Universitario Marques de Valdecilla, Santander, Spain; Uppsala University Hospital, Uppsala, Sweden; Royal Prince Alfred Hospital, Camperdown, Australia; Royal North Shore Hospital, St Leonards, Australia; F Hoffmann-La Roche Ltd, Basel, Switzerland; Nottingham University Hospitals, Nottingham, United Kingdom

**Background:** The use of trastuzumab (H)-based therapy for HER2-positive BC has significantly altered outcomes. Yet up to 64% of metastatic patients (pts) fail to respond (Robert JCO 2006) and most pts will progress within 24-42 months (m) following an initial response for metastatic disease. Preclinical data suggest several in vitro resistance mechanisms but confirmatory in vivo data are lacking, preventing optimal personalized care. This ongoing proof-of-concept study examines serial bx in pts with HER2-positive metastatic BC in order to assess BM profiles across multiple lines of treatment.

**Methods:** Key eligibility criteria include: centrally confirmed HER2 status, minimum of 1 disease site considered suitable for serial bx, normal coagulation profile and cardiac function, prior adjuvant/neoadjuvant taxane and H completed ≥12 m and ≥6 m respectively, ECOG ≤2. Pts receive q3wk H with clinician choice of taxane (docetaxel 75-100 mg/m² q21, paclitaxel 80 mg/m² weekly or 175 mg/m² q21 [TH]). At the time of progressive disease, pts receive capecitabine (X) and H. Two core bx and 1 optional fine needle aspirate are performed at the following times: baseline (after 3 weeks [w] of TH), at 6 w, at time of first progression prior to XH, and at the time of progression on XH. Tumor assessments are performed at 6 w, then 9 w intervals, with cardiac assessment every 6 m. Primary endpoint aims to explore and potentially define BM signatures that could alter during HER2-targeted therapy and predict for decreased or increased sensitivity to H-based treatment. Secondary endpoints include bx safety, ORR and TTP. At time of baseline bx, pts have the option to complete a pt satisfaction questionnaire.

**Results:** Between August 2009 and June 2011, 58 pts were screened; 29 enrolled. The other 29 pts were screen failures, with 3 pts (5%) specifically declining entry due to the requirement for serial bx. Median age is 53 y (range 37-86), with 9 and 20 pts having recurrent or de novo metastatic disease. Eight pts received adjuvant systemic treatment, including H in 4 pts. Baseline bx performed in the breast, n (%): 15 (52), bone: 6 (21), liver: 4 (14), and lymph nodes: 4 (14); 14 and 27 pts underwent bx at 3 and 6 w respectively. After a median of 34 w of treatment 11 pts progressed on TH and 7 pts underwent planned bx. There were 12 SAEs, none related to bx. Most AEs were grade 1/2 (95%) with 3% grade 3 and 1 death due to intercurrent illness. From a total of 43 bx, 12 (27%) grade 1/2 AEs (hematoma 2, transient hypotension 2, mild pain 8) were reported, with resolution in all instances. Fifteen pts consented to the pt satisfaction substudy.

**Conclusions:** Preliminary results of the first reported serial bx study in metastatic BC demonstrated 95% pt acceptance of this approach. Evaluation of BM profiles will be conducted following planned recruitment of 50 pts. To date, the feasibility and safety of obtaining serial bx in metastatic BC is supported by the current safety profile and patient uptake. Updated recruitment data will be presented.

**P5-23-01**

The Impact of Primary Tumor Resection on the Survival of Patients with Stage IV Breast Cancer According to Molecular Subtype.

Ahn SK, Moon H-G, Kim JS, You JM, Shin HC, Han W, Noh D-Y. Seoul National University Hospital

**Purpose:** The main treatment for stage IV breast cancer is currently systemic therapy. Surgical resection of the primary tumor is usually done for treating the tumor-related complications. Recent studies have suggested that surgery may improve the long-term survival of stage IV breast cancer patients. We evaluated the impact of the primary surgical resection site on the survival of stage IV breast cancer patients according to molecular subtype using nationwide Korean breast cancer registry data.

**Methods:** We analyzed the records of the stage IV breast cancer patients from Korean Central Cancer Registry (KCCR) between 1999 and 2008. We used clinical assays to distinguish luminal A (HR+/HER2−, n=290), luminal B (HR+/HER2+, n=154), Basal-like (HR-/HER2−, n=107) and HER2 (HR-/HER2+, n=145). The clinical and tumor characteristics, the type of treatments and the overall survival were compared between the surgically versus nonsurgically treated patients according to molecular subtype.

**Results:** Of the 1091 identified patients, 719 (65.9%) received surgical excision of their primary tumor and 372 (34.1%) did not. The mean survival was 86 months versus 43 months for the surgically treated patients vs. the patients without surgery, respectively (p<0.001). On a multivariate analysis with using the Cox model and after adjusting for tumor size, visceral metastases, the number of metastatic sites and bone only metastases, surgery was an independent factor for improved survival in patients with luminal A type (Hazard Ratio, 0.505; 95% Confidence Interval, 0.265-0.962, p=0.038) but not in those with luminal B (p=0.192), basal-like (p=0.128) and HER2 subtype (p=0.114). **Conclusion:** Surgical resection of the primary tumor in stage IV breast cancer patients was independently associated with improved survival only in luminal A subtype.
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P5-23-02
Clinicopathological Features of Young Patients Age <35 Years with Breast Cancer in Japan.

Kataoka A, Tokunaga E, Masuda N, Shien T, Ohno S, Kinoshita T, Shimizu C. Breast Surgery Clinic, Minato-ku, Tokyo, Japan; Kyushu University Hospital, Fukuoka City, Fukuoka, Japan; National Hospital Organization Osaka National Hospital, Osaka City, Osaka, Japan; Okayama University Hospital, Okayama City, Okayama, Japan; National Hospital Organization Kyushu Cancer Center, Fukuoka City, Fukuoka, Japan; National Cancer Center Hospital, Chuo-ku, Tokyo, Japan; The Japanese Breast Cancer Society (JBCS) Study Group on Breast Cancer in Young Women

Background: The aim of this study is to clarify clinicopathological features of breast cancer in young women.

Materials and methods: Clinicopathological characteristics were compared between young (<35) patients and non-young (≥35) patients among 109,617 records of JBCS database registered from 2004 to 2009, and overall survival (10-yr OS) were calculated among 146,690 records from 1975 to 2000 with 8.6 years of median follow-up period. Results: Clinicopathological factors of 2,982 young patients (2.7%) were compared with 106,295 non-young patients. Young patients had more familial history of breast cancer, more subjective symptom, less bilateral tumor, lower tumor size, more inflammatory breast cancer, more positive node, less ER-positive, more HER2-positive, more triple-negative tumor, and more advanced TNM-stage.

Comparison of clinicopathological factors between young and non-young patients with breast cancer

<table>
<thead>
<tr>
<th>Factors</th>
<th>Young Patients (&lt;35)</th>
<th>Non-Young Patients (≥35)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Family history of breast cancer</td>
<td>12.4%</td>
<td>9.4%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Subjective symptom</td>
<td>83.2%</td>
<td>87.3%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Bilateral breast cancer</td>
<td>2.8%</td>
<td>7.2%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BMI &gt;18</td>
<td>11.4%</td>
<td>5.2%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BMI &lt;25</td>
<td>10.4%</td>
<td>22.8%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Tumor size (mean)</td>
<td>2.9cm</td>
<td>2.5cm</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Lymph node metastasis</td>
<td>35.2%</td>
<td>31.2%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Inflammatory breast</td>
<td>1.1%</td>
<td>8.6%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ER-positive</td>
<td>72.7%</td>
<td>76.9%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HER2-positive</td>
<td>18.2%</td>
<td>15.8%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Triple-negative</td>
<td>16.3%</td>
<td>12.9%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>TNM Stage I</td>
<td>42.4%</td>
<td>50.1%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>TNM Stage II</td>
<td>43.9%</td>
<td>39.8%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>TNM Stage III</td>
<td>10.4%</td>
<td>7.8%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>TNM Stage IV</td>
<td>3.3%</td>
<td>2.4%</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Young patients were received more neoadjuvant chemotherapy and breast conserving therapy (BCT), compared with non-young patients.

Comparison of treatments between young and non-young patients with breast cancer

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Young Patients (&lt;35)</th>
<th>Non-Young Patients (≥35)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adjacent therapy</td>
<td>24.7%</td>
<td>11.3%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Neoadjuvant therapy</td>
<td>89.1%</td>
<td>89.8%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Neoadjuvant + chemotherapy</td>
<td>15.7%</td>
<td>16.9%</td>
<td>0.4085</td>
</tr>
<tr>
<td>Neoadjuvant + radiation therapy</td>
<td>11.9%</td>
<td>11.6%</td>
<td>0.4085</td>
</tr>
<tr>
<td>Neoadjuvant + radiation therapy</td>
<td>1.2%</td>
<td>1.1%</td>
<td>0.7402</td>
</tr>
<tr>
<td>Surgery</td>
<td>98.6%</td>
<td>98.8%</td>
<td>0.2604</td>
</tr>
<tr>
<td>Mastectomy</td>
<td>55.0%</td>
<td>41.9%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Adjuvant therapy</td>
<td>79.1%</td>
<td>80.6%</td>
<td>0.006</td>
</tr>
<tr>
<td>Adjuvant + chemotherapy</td>
<td>55.5%</td>
<td>41.5%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Adjuvant + endocrine therapy</td>
<td>72.6%</td>
<td>81.2%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Adjuvant + radiation therapy</td>
<td>62.9%</td>
<td>50.9%</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Eighty-percent of patients were received adjuvant therapy, among them, treatment rate of chemotherapy, molecular targeted therapy and radiation therapy was significantly higher in young patients than non-young patients. There was significant difference in 10-yr OS between young and non-young patients with Stage I-IIIA disease (Stage I 80% vs. 90%, IIA 78% vs. 85%, IIB 67% vs. 78%, IIC 42% vs. 64%), but not with Stage IIB and IIC. Advanced TNM stage, lymph node metastasis and ER-negative tumor were significantly poorer prognostic factors for young patients by univariate analysis. Approximately 9% of young patients were at pregnancy at the time of diagnosis had more advanced disease and worse prognosis.

Conclusions: We conclude that young patients with breast cancer have an advanced or an endocrine-unresponsive tumor and have unfavorable outcome. Advocacy for awareness of breast cancer to young women and development of new targeted therapy against advanced, HER2-positive and triple-negative tumor are important to improve the survival of young patients with breast cancer.

P5-23-03
Breast Cancer in Elderly Treatment Algorithm – A New Approach To Optimize the Management of Breast Cancer in Older Patients.

Tahir M, Pretorius R, Robinson T, Walker R, Stotter A. University Hospitals of Leicester NHS Trust, Leicester, United Kingdom

Background: Elderly patients have been receiving a sub-standard treatment for early breast cancer when compared to younger age group patients. A higher percentage of them are being treated with primary endocrine therapy (PET) based on the premise that they may not survive surgery or live long enough to benefit from it. This clinical practice, however, there is no validated assessment in place to assess their surgical risk or estimate their life expectancy. This study aimed to optimize the treatment of early breast cancer in older patients using comprehensive geriatric assessment (CGA). It tested the hypothesis that CGA could be used to predict 2-year survival in older breast cancer patients. Based on that a treatment algorithm was devised which could be used to recommend whether PET or surgery plus endocrine treatment would be best indicated in individual patients.

Methods: The study included women >70 years of age with early breast cancer, seen in a dedicated Leicester clinic between 04/2005 and 04/2007. All patients had comprehensive assessment including documentation of co-morbidities, Mini-mental state examination (MMSe), Geriatric depression score (GDS), Activities of daily living (ADL), Instrumental activities of daily living (IADL) and American society of anesthesiologist score (ASA). Analysis was performed to find components of the CGA that were helpful in predicting 2-year survival in these patients.

Results: 123 patients were included; age range was 70-94 (median-82). Twenty-two patients died within 2-years. Logistic regression analysis found MMSe, ADL, and ASA score to have an independent association with 2-year survival. A statistically significant correlation (p-value 0.000) was found between the dichotomized combined-score of these components and 2-year survival. Breast Cancer in Elderly Treatment Algorithm (BCETA) was devised using the scores of these three components. Patients who scored one or higher (high-risk group), were found to have <48% two-year survival; those, who scored zero (low-risk group), have >60% chance of two-year survival. The overall accuracy for the algorithm was 81%.

Conclusion: Breast Cancer in Elderly Treatment Algorithm is a new and systematic approach to optimize the management of breast cancer in elderly patients. It is helpful in identifying high-risk patients with expected short-survival who may benefit from PET, if their cancer is hormone receptor positive. Patients with predicted longer life expectancy (low-risk) may be recommended standard treatment. A prospective study is ongoing at Leicester research clinic to validate the results.
P5-23-04
Cruz MRS, Motta E, Silva EMK, Bernardo SG, Atallah AN. Hospital Sao Jose, Sao Paulo, Brazil; Universidade Federal de Sao Paulo, Sao Paulo, Brazil

Background: Breast cancer, the most common female malignancy, is also the most frequent tumor in women of reproductive age. Young women with breast cancer usually present adverse prognostic features and higher rates of recurrence when compared with their older counterparts. Adjuvant chemotherapy is usually recommended for this group of patients and premature ovarian failure (POF) is a potential complication.

Material and Methods: We conducted a systematic search in MEDLINE, EMBASE, Cochran Systematic Reviews databases and ClinicalTrials.gov from their inception until June 2011. The search was conducted to include published peer-reviewed studies on the issue of fertility preservation in women with breast cancer undergoing adjuvant chemotherapy. The search terms were tailored individually for each database to include the following Medical Subject Headings (MeSH) and text words: gonadotropin releasing hormone, breast neoplasms, drug therapy, premature ovarian failure and fertility. No language restriction was applied. We also searched for additional studies by reviewing the reference lists of retrieved articles, and studies presented in abstract form were also located in this manner. The main outcomes were return of spontaneous menstruation, ovulation and pregnancy rate.

Results: We found 8 RCTs that met the inclusion criteria. Data were extracted from 3 RCTs. Data were only available for resumption of ovulation. There were no data available for markers of ovarian function, rate of ovulation and pregnancy. The rate of women with spontaneous menstruation demonstrated a statistically significant difference in favor of the use of GnRH-a (OR 3.11; 95% CI, 2.04–4.72).

Discussion: Use of GnRH concomitant to adjuvant chemotherapy in premenopausal breast cancer women aiming to reduce rates of POF has been subject of controversy during the last decade. Evidence from recently presented RCTs suggests there is a benefit of GnRH for ovarian function protection in this setting.

P5-23-05
Withdrawn by Author

P5-23-06

Aim: To describe the effects of the development, implementation and prospective systematic evaluation and adaptation of a clinical care pathway for the management of patients with early breast cancer between 2002 and 2010) in a single breast unit.

Materials and methods: In 2002 a clinical pathway was developed by the multidisciplinary breast team of the Sint Augustinus Hospital for de diagnosis and treatment of patients with operable breast cancer. Performance measurements were documented systematically by care providers using an order communication, planning and result reporting system. Annual analysis of predefined clinical outcome measures and indicators was performed. Based on these data and evidence based guidelines the pathway was regularly adapted to improve patient care.

Results: The annual number of patients included in the pathway (289 vs 390, p 0.01), proportion of patients with Tis-T1 tumors (42% vs 58 %, p 0.01), negative lymph nodes (44% vs 58%, p < 0.01) and no metastases at diagnosis (91.5% vs 95.9%) has risen significantly between 2002 and 2010. Histological subtypes remained the same. The average length of hospital stay (7.0 days vs 4.1 days, p 0.01) nearly halved and the proportion of breast conserving surgery (BCS) (43% vs 57%), preoperative guide wire localization (14% vs 27%) for impalpable lesions and use of sentinel node biopsy (0% vs 49%) increased significantly (p 0.01). Evolution of quality indicators defined by Eusoma (www.eusomadb.org/indicators.htm) between 2002 and 2010 shows a significant improvement of cancer care: proportion of positive of preoperative histologic diagnosis (59.7% vs 88.4%, p 0.001), more then 9 lymph nodes removed when axillary clearance performed (85.6 vs 91.4%, p< 0.04), BCS for invasive carcinoma up to 3 cm (62.0% vs 82.6%, p 0.016), BCS for DCIS up to 20 mm (43.8% vs 78.6%, p 0.016), hormone therapy in endocrine sensitive tumor (84.8% vs 97.4%, p 0.002), adjuvant chemotherapy in ER negative (PT1c or N+) invasive carcinoma (72% vs 95.6%, p 0.028), proportion of second surgery (25% vs 10%, p 0.001) and clear margins after last operation (95% vs 99%, p 0.02). All mandatory EUSOMA requirements were fulfilled in 2010. Patient satisfaction improved significantly over the years (13/19 measured parameters p <0.05 between 2002-2010). Progression free 4 year survival was significantly higher for all patients, for T1 tumors only and for T2-T4 tumors only, treated in 2006-2008 compared to 1999-2002 and 2003-2005 (respectively p 0.006, p 0.05, p 0.06). Overall 4 year survival of the entire M0 population treated in 2006-2008 was significantly better (p 0.05)

Conclusion: Although the patient characteristics changed over the years due to better screening, this clinical pathway for the treatment of patients operable breast cancer proved to be an important tool to improve the quality of patient care and patient satisfaction. Better adherence to guidelines and constant feedback of treatment data to the breast team contributes to a superior patient outcome. Measuring quality indicators proved useful to develop quality measures improving patient care.
P5-23-07
Treatment Strategy of Locally Advanced Breast Cancer in Sub Group (T3N1M0, T4bN1M0) in Developing Countries.
Gupta AK, Kaushal M. Mahatma Gandhi Memorial Medical College and Maharaja Yashwantrao Hospital, Indore, Madhya Pradesh, India

With the recent advances in modern day chemotherapy and hormonal therapy for locally advanced breast cancer [stage III, T3 N1 and T4bN1], most of the oncologist around the globe now prefer to give neoadjuvant chemotherapy and, or hormonal therapy followed by surgery in the treatment of these cases.

Randomized research have also shown that this strategy provides ‘pathological complete resolution’ as compared to surgery and then adjuvant chemotherapy in terms of decreasing in size of tumor, decrease in vascularity of tumor mass and micrometastasis. Some author believes that this improve the overall survival duration of patients.

This protocol is widely accepted but … BUT WHAT IF YOU LOSE YOUR PATIENT ON FOLLOWUP …

Especially in the developing countries of south east asian region and African continent, where there is poor socioeconomic status and lack of education among common people. The patient gets frustrated after first few doses of neoadjuvant therapy, due to side effects of chemotherapy and don’t consider chemotherapy as a treatment compare to surgery. Thus the patient loses faith in treatment strategy itself and go unretraceable after one or two sessions of the therapy.

The government of developing countries lack resources in terms of manpower and money for satisfactory completion of treatment making the above condition even worse. In our study we followed 230 patients of locally advanced breast cancer in subgroup of stage III [T3 N1, T4bN1] and divided them into two plans on random basis.

In first plan we approached with the surgical management followed by adjuvant chemotherapy & counseled the patients for completion of chemotherapy. This plan was shown to 115 patients, out of which 100 patients provided good acceptance & completed the course of chemotherapy. There were 15 patients who remained unretraceable after surgery.

On the other hand in the 2 group the patients were counseled for neoadjuvant chemotherapy and then surgery in next stage. In this group 115 patients were introduced, out of which 60 patients were lost on follow up after 2-3 sessions. The majority of patients complained about the side effects of chemotherapy and financial reasons and did not come back. Rest of the patients completed the whole course.

With no doubt about the result of the treatment, the second group has equivocal survival advantages in terms of morbidity and mortality, but we lost more than half of the patients of second group before completion of treatment.

Primarily the acceptance of surgical treatment is more in developing countries, as it is quite more feasible for government and even economical for common people. Thus we prefer the strategy of surgery followed by adjuvant chemo/hormonal therapy in locally advanced breast cancer [stage III T3N1, T4bN1] especially in developing countries like India.

THE MOTTO OF THIS STRATEGY IS TO PROVIDE BENEFIT OF COMPLETE TREATMENT TO THE PATIENT THAN TO LOSE THE PATIENT COMPLETELY BEFORE TREATMENT.

OT1-01-01
Prospective Clinical Trial Evaluating Efficacy of Zoledronic Acid (ZA) Prophylaxis for Prevention of Aromatase Inhibitor Associated Musculoskeletal Symptoms: ZAP-AIMSS Trial.
Bardia A, Blackford A, Jeter S, Tarpinian K, Fetting JF, Miller R, Slater S, Henry NL, Giles J, Stearns V. Johns Hopkins Kimmel Cancer Center, Baltimore, MD; University of Michigan, Ann Arbor, MI; Columbia University, New York City, NY

Brief background: Aromatase inhibitor associated musculoskeletal symptoms (AIMSS) occur in approximately 50% of patients receiving AIs (Henry 08). However, interventions to prevent or treat AIMSS have not been established. In a retrospective study (Muslimani 09), patients receiving bisphosphonates along with AIs were less likely to report AIMSS compared to those not taking bisphosphonates (35% vs. 60%). However, the efficacy of bisphosphonates in reducing incidence of AIMSS has not been studied prospectively, so it cannot be recommended for routine clinical practice.

Trial design: We are conducting a single arm, phase II clinical trial of 4 mg intravenous zoledronic acid (ZA) given at baseline and at 6 months, in combination with letrozole 2.5 mg daily for one year.

Development of AIMSS will be assessed using the standardized Health Assessment Questionnaire (HAQ-DI) and pain Visual Analog Scale (VAS) at baseline, 1, 3, 6, and 12 months. Secondary endpoints include mammographic breast density (when intact contralateral breast), bone mineral density, bone turnover metabolites, circulating inflammatory markers, and patient reported quality of life measures.

Prevalence of AIMSS will be compared to historical controls from a recently completed multi-institutional study designated Exemestane and Letrozole Pharmacogenetics (ELPh trial, ClinicalTrials.gov #NCT00228956). The current study has the same eligibility criteria, method and intervals of outcome assessment, and AI medication, as the ELPh trial, ensuring that the two cohorts are comparable.

Eligibility criteria: Postmenopausal women who have completed local therapy and chemotherapy for hormone receptor positive DCIS or stage I-III breast cancer and who are scheduled to receive adjuvant AI. Prior tamoxifen therapy is permitted.

Specific aims:
1. Percentage of women experiencing AIMSS at 1, 3, 6, and 12 months after initiation of ZA and letrozole, as compared to historical controls.
2. Change in bone mineral density and breast density between baseline and 12 months for those receiving ZA and letrozole, as compared to historical controls.
3. Change in bone turnover markers and inflammatory markers between baseline and 1, 3, 6 and 12 months for those receiving ZA and letrozole, as compared to historical controls.

Statistical methods: Allowing for a 20% dropout rate, a total sample size of 59 patients yields 80% power to detect reduction in AIMSS incidence from 50% to 30% with a two sided type I error rate of 5%. The rates of AIMSS and other endpoints at each time point and across all time points between controls and patients will be compared with a logistic regression model that adjusts for potential confounding variables and include random effects as appropriate to account for correlation between outcomes in the same patient.

Present accrual and target accrual: The Johns Hopkins Institutional Review Board approved the study and it opened to accrual in January 2011. Since that time, 12 participants have signed consent and started therapy, and 2 have completed the 3 month evaluation.

Funding: Trial supported by BCRF. ZA and letrozole kindly supplied by Novartis.
OT1-01-02
A Multicentre Study Assessing 12-Weekly Intravenous Bisphosphonate Therapy in Women with Low Risk Bone Metastases from Breast Cancer – The TRIUMPH Trial. Bougainin N, Hilton J, Vandermeer L, Hopkins S, Spencer P, Robbins D, Amir E, Dent S, Milano C, Oui D, Dranitis G, Clemens M. The Ottawa Hospital Cancer Center; Ottawa, ON, Canada; Princess Margaret Hospital, Toronto, ON, Canada; The Ottawa Hospital, Ottawa, ON, Canada

Background: Metastatic bone disease is a major cause of morbidity and mortality for breast cancer patients. Bisphosphonates (BP) have been shown to significantly delay the onset and frequency of skeletal related events (SREs), improve pain control and overall quality of life. Most patients receive intravenous BP every 3-4 weeks regardless of their individual risk for a SRE. This “one size fits all” strategy could expose those patients at a relatively low risk of SREs to an increased chance of adverse drug effects, as well as to the financial and quality of life burden of multiple visits to the cancer centre for treatments. This study aimed to assess whether IV BP can be safely given at reduced frequency.

Methods: The primary objective of this study is to demonstrate in women with biochemically defined low-risk bone metastases that the administration of IV BP every 12 weeks is sufficient to maintain biochemical stability for one year. Eligibility criteria include; bone metastases from breast cancer, have received at least three months of regular 3-4 weekly IV BP, satisfactory renal function, adequate dental health, no systemic treatment change or recent SRE within 4 weeks of study entry. Low risk disease will be defined as serum CTx levels <600 ng/L. Biochemical failure is defined as CTx levels >600 ng/L measured at predefined time points (6, 12, 24, 36 and 48th). Secondary objectives are to evaluate the palliative benefit of 12-weekly IV BP therapy as reflected by occurrence of SREs, analgesic use, self-reported pain using the validated BP and FACT-BP questionnaires. Sample size was calculated at 68 patients. Given the small sample size, nonparametric Bootstrapping will be employed to calculate point estimates, standard deviations and 95% confidence intervals (CIs). An exploratory multivariable analysis will also be undertaken to determine baseline factors that were associated with patient’s maintaining their telopeptide levels in the low risk range.

Conclusion: TRIUMPH opened in October 2010 and as of June 2011, has quickly accrued 54/68 patients (79%). This trial has the potential to allow lower risk women to receive less frequent dosing of bisphosphonates, thus improving their quality of life with less cancer related events (SREs), improve pain control and overall quality of life. Most patients receive intravenous BP every 3-4 weeks regardless of their individual risk for a SRE. This “one size fits all” strategy could expose those patients at a relatively low risk of SREs to an increased chance of adverse drug effects, as well as to the financial and quality of life burden of multiple visits to the cancer centre for treatments. This study aimed to assess whether IV BP can be safely given at reduced frequency.

OT1-01-04
NEO-ZOTAC: A Phase III Randomized Trial with Neoadjuvant Chemotherapy (TAC) with or without Zoledronic Acid for Patients with HER2-Negative Large Resectable or Locally Advanced Breast Cancer.

van de Ven S, Nortier JW, Liefers GJ, ten Tije A, Kessels LW, van Laarhoven HWM, van Warmenhad LJC, Vriens B, van den Bosch J, van Meersch-Klein Kranenburg E, van Leeuwen E, Kroep JR. Leiden University Medical Center. Leiden, Netherlands; Amphia Hospital, Breda, Netherlands; Deventer Hospital, Deventer, Netherlands; University Medical Center St. Radboud Nijmegen, Nijmegen, Netherlands; Catharina Hospital, Eindhoven, Netherlands; Academic Hospital Maastricht, Maastricht, Netherlands; Albert Schweitzer Hospital, Dordrecht, Netherlands; BOOG Study Center, Amsterdam, Netherlands

Background: The role of bisphosphonates (BPs) when added to the preoperative chemotherapy (TAC) regimen is unexplored in the neoadjuvant setting for breast cancer. Zoledronic acid is approved in the USA, Canada, and European countries for the treatment of bone metastases from solid tumors. In patients with castrate-resistant prostate cancer, denosumab significantly improved bone metastasis-free survival (BMFS) compared to placebo. The D-CARE trial evaluates BMFS effects of denosumab in women with stage II or III breast cancer.

Methods: Women with node-positive or locally advanced (T3 or T4) disease, and known hormone and HER-2 receptor status are eligible. Standard-of-care adjuvant or neoadjuvant chemo-, endocrine, or HER-2 targeted therapy, alone or in combination must be planned with curative intent. Women with a prior history of breast cancer (other than ductal carcinoma in situ [DCIS] or lobular carcinoma in situ [LCIS]) or distant metastasis, oral bisphosphonate (BP) use within 1 year or any intravenous BP use are excluded. Patients are randomized 1:1 to receive denosumab 120 mg or placebo subcutaneously monthly for 6 mos, then every 3 mos, for a total of 5 yrs treatment. All patients receive vitamin D (≥ 400 IU) and calcium (≥ 500 mg) supplements. Primary endpoint of this event-driven trial is BMFS. Secondary endpoints include disease-free (DFS) and overall survival. The study is powered for both, BMFS and DFS. Safety, quality of life assessments and biomarkers are additional endpoints. The trial, sponsored by Amgen Inc. and registered with the ClinicalTrials.gov identifier NCT01077154 began enrolling patients in June 2010. PG and DF are supported in part by the Avon Foundation, NY.

OT1-01-03
A Phase 3 Randomized, Double-Blind, Placebo-Controlled Multicenter Study Comparing Denosumab with Placebo as Adjuvant Treatment for Women with Early-Stage Breast Cancer Who Are at High Risk of Disease Recurrence (D-CARE).

Goss PE, Barrios CH, Bell R, Finkelstein D, Iwata H, Martin M, Braun A, Ke C, Maniater T, Braun S, Dansy R, Coleman RE. Massachusetts General Hospital Cancer Center; Boston, MA; PUCRS School of Medicine, Porto Alegre RS, Brazil; Barwon Health, Geelong, Victoria, Australia; Atchi Cancer Center Hospital, Nagoya, Japan; Hospital Gregorio Maranon, Madrid, Spain; Amgen Inc. Thousand Oaks, CA; Angen (Europe) GmbH, Zug, Switzerland; University of Sheffield, Sheffield, United Kingdom

Background: Bone is a common site of distant recurrence in women with early-stage breast cancer. Cancer cells are thought to stimulate osteoclast-mediated bone resorption, which releases growth factors and cytokines that promote tumor growth. RANK Ligand (RANKL) is the key mediator of osteoclast-induced bone destruction. In preclinical studies, RANKL inhibition reduced the incidence of bone and lung metastases, suppressed tumor progression, and prolonged survival of tumor-bearing mice. Effects were additive with hormonal, chemotherapeutic, or targeted therapies. Denosumab is a fully human monoclonal antibody against RANKL, approved in the U.S. for the prevention of skeletal-related events in patients with bone metastases from solid tumors. In patients with castrate-resistant prostate cancer, denosumab significantly improved bone metastasis-free survival (BMFS) compared to placebo. The D-CARE trial evaluates BMFS effects of denosumab in women with stage II or III breast cancer.

Methods: Women with node-positive or locally advanced (T3 or T4) disease, and known hormone and HER-2 receptor status are eligible. Standard-of-care adjuvant or neoadjuvant chemo-, endocrine, or HER-2 targeted therapy, alone or in combination must be planned with curative intent. Women with a prior history of breast cancer (other than ductal carcinoma in situ [DCIS] or lobular carcinoma in situ [LCIS]) or distant metastasis, oral bisphosphonate (BP) use within 1 year or any intravenous BP use are excluded. Patients are randomized 1:1 to receive denosumab 120 mg or placebo subcutaneously monthly for 6 mos, then every 3 mos, for a total of 5 yrs treatment. All patients receive vitamin D (≥ 400 IU) and calcium (≥ 500 mg) supplements. Primary endpoint of this event-driven trial is BMFS. Secondary endpoints include disease-free (DFS) and overall survival. The study is powered for both, BMFS and DFS. Safety, quality of life assessments and biomarkers are additional endpoints. The trial, sponsored by Amgen Inc. and registered with the ClinicalTrials.gov identifier NCT01077154 began enrolling patients in June 2010. PG and DF are supported in part by the Avon Foundation, NY.
Eligibility criteria: Main inclusion criteria are large resectable or locally advanced breast cancer (T2, T3, T4, every N, M0), measurable disease, histological proven HER2-negative breast cancer, age ≥18 years, WHO 0-2, adequate bone marrow-, renal- and liver function, written informed consent. Main exclusion criteria are evidence of distant metastases (M1), history of breast cancer, prior breast surgery, prior chemotherapy or radiation therapy, previous malignancy within 5 years, prior bisphosphonate usage, peripheral neuropathy > grade 2, current active dental problems.

Study endpoints: The primary endpoint of this study is the pathologic complete response (pCR) rate to neoadjuvant chemotherapy with or without zoledronic acid at surgery. Secondary endpoints are clinical response (RECIST 1.1), ER/PR and HER2 heterogeneity in core biopsy vs. operation specimen, toxicity, disease free survival and overall survival.

Optional side studies include fluorescent imaging (SoftScan®), changes in bone biochemical markers and the insulin-like growth factor (IGF) pathway, circulating tumor cells (CTC's) and circulating endothelial cells (CEC's), the false-negative rate of the sentinel node biopsy after neoadjuvant chemotherapy, single nucleotide polymorphisms (SNPs) and Ki-67, apoptotic index and IGF pathway in core biopsy and operation specimen.

Statistical Methods: This study is designed as a randomized, open-label, multi centre phase III trial. It is anticipated that using a 5% significance level based on the two-sided Fisher’s exact test with a power of 80%, a total number of 250 patients (125 patients in each arm) are needed to show an improvement of the pCR rates from 17% in arm B to 34% in the experimental arm A. Randomization will be done according to the Pocock’s minimization technique stratified by cT-classification, cN-classification and estrogen receptor status. The primary endpoint will be analyzed using the Cochran-Mantel-Haenszel test.

An interim efficacy analysis (analyzing pCR) after 100 operated patients is planned.

Accrual: Patients are currently being included from 27 centers in the Netherlands. Presently (16th June 2011) a total number of 116 patients have been included since start of the study (July 2010). The expected end of accrual of 250 patients will be the last quarter of 2012.

OT1-02-01
Phase II Neoadjuvant Trial of Anthracycline Based Regimens Followed by a Combination with Nanoparticle Alummin-Bound Paclitaxel and Trastuzumab in Patients with Operable T2-3,N0-1,Her2 Positive Breast Cancer.
Iwamoto M, Takahashi Y, Kimura K, Tanaka S, Uchiyama K. Osaka Medical College, Osaka, Japan

Background: Anthracycline and taxane have been widely used and studied in neoadjuvant setting for treatment of locally advanced breast cancer. Various regimens have explored the addition of newer agents to determine safety and efficacy, and pathological complete response(pCR) has been demonstrated to be associated with favorable overall survival in primary breast cancer. In addition, three year median follow-up data of the TECHNO Trial revealed that the neoadjuvant combination of trastuzumab and chemotherapy resulted in a high chance for a pCR. We conducted a clinical Phase II neoadjuvant trial of Anthracycline based regimens(EC,AC,FEC) followed by a combination with nanoparticle albumin-bound Paclitaxel and Trastuzumab in patients with operable T2-3,N0-1,Her2 positive breast cancer.

Patients and Methods: The study is designed to evaluate EC or AC or FEC followed a combination of nanoparticle albumin-bound Paclitaxel and Trastuzumab as neoadjuvant therapy in patients with Her2 positive locally advanced(T2-3,N0-1) breast cancer. Patients are treated with neoadjuvant EC(Epirubicin 90 mg/m², Cyclophosphamide 600 mg/m²) or AC(Doxorubicin 90 mg/m², Cyclophosphamide 600 mg/m²) or FEC(Fluorouracil 500mg/m², Epirubicin 100 mg/m², Cyclophosphamide 500 mg/m²) q21d x 4, followed by a combination with nanoparticle albumin-bound Paclitaxel(260 mg/m²) and Trastuzumab q21d x 4.

Patients undergo surgery 4-6 weeks later from completing chemotherapy. pCR, the primary endpoint is defined as no evidence of invasive tumors in the final surgical sample both in the breast and axillary lymph nodes. Secondary endpoints include objective clinical response rate, disease-free interval, overall survival, rate of breast concerning surgery, and the safety of the treatment.

HALT MBC: HER2 Suppression with the Addition of Lapatinib to Trastuzumab in HER2-Positive Metastatic Breast Cancer (LPT112515).
Lin N, Danso M, David A, Mascato J, Ellis C, DeSilvio M, Garofalo A, Nagarwala Y, Winer E. Dana-Farber Cancer Institute; Virginia Oncology Associates; Augusta Oncology Associates; Missouri Cancer Associates; GlaxoSmithKline

Background
Lapatinib in combination with trastuzumab enhanced anti-tumor activity in HER2-positive breast cancer (BC) preclinical models. In patients (pts) with trastuzumab-treated, HER2-positive metastatic (M) BC, treatment with the combination was associated with longer progression-free (PFS) and overall survival (OS) compared with lapatinib alone. In pts with stage II/III BC, preoperative treatment with the combination plus paclitaxel resulted in significantly higher pathological complete response rates compared with paclitaxel combined with either agent alone. This evidence supports the concept of dual HER2 blockade as a treatment strategy for HER2-positive BC. This present study is designed to evaluate whether the addition of lapatinib improves PFS among women with HER2-positive MBC receiving trastuzumab as maintenance therapy.

Trial Design
In this open-label, Phase III study, pts are stratified by line of treatment (first/second) and hormone receptor status (positive/negative) then randomized 1:1 to receive maintenance treatment with either lapatinib (1000mg once daily, continuously) in combination with trastuzumab (6mg/kg once every 3 weeks [Q3W]) or trastuzumab (6mg/kg Q3W) alone. Pts will receive study treatment until disease progression, death, discontinuation due to adverse events or other reasons.

Eligibility Criteria
Pts with HER2-positive MBC who have completed 12-24 weeks of first- or second-line treatment with trastuzumab plus chemotherapy and have objective response or stable disease. Pts with stable brain metastasis are eligible if entering the study on second-line treatment.

Specific Aims
The primary objective is to compare PFS of lapatinib in combination with trastuzumab to trastuzumab as continued HER2 suppression therapy. Secondary objectives are to evaluate OS, clinical benefit rate, safety and tolerability.

Statistical Methods
Efficacy endpoints will be analyzed in the intent to treat population. A total of 193 PFS events from 280 randomized pts will be required to detect a 50% increase in median PFS in pts who receive lapatinib
plus trastuzumab compared with trastuzumab (median PFS time is 27 versus 18 weeks, respectively); hazard ratio of 0.67 with an 80% power and a 1-sided type I error of 0.025.

Present and Target Accrual

Sixteen of the target 280 pts have been randomized. The trial is currently open for accrual in the United States and Canada.

OT1-02-03

EGF114299: Safety and Efficacy of an Aromatase Inhibitor (AI) in Combination with Lapatinib (L), Trastuzumab (T) or Both for the Treatment of Hormone Receptor-Positive (HR+), HER2+ Metastatic Breast Cancer (MBC).

Johnston S, Leigh M, Florance A, Wroblewski S, Gradishar W. Royal Marsden NHS Foundation Trust & Institute of Cancer Research; GlaxoSmithKline, Collegeville, PA; GlaxoSmithKline, Research Triangle Park, NC; Northwestern University

Background: Data from the L plus letrozole (EGF30008) and the Trastuzumab and Anastrozole Directed Against ER-Positive HER2-Positive Mammary Carcinoma (TaNDEM) studies demonstrated that HER2 targeted and endocrine therapy (ET) is a better strategy than ET alone. In EGF30008, progression-free survival (PFS) in the HER2+ population was statistically significantly longer in the letrozole plus L group compared with the letrozole plus placebo group. The HR was 0.71 (95% CI: 0.53-0.96; stratified log rank \( P < 0.019 \)). Median PFS was 35.4 weeks in the letrozole plus L group compared with 13.0 weeks in the letrozole plus placebo group.

The present study (EGF114299) is a phase III, open-label, multicenter trial designed to evaluate benefit in overall survival (OS) provided by L/T/AI and L/AI, in pts with HR+/HER2+ MBC who have received neo-/adjuvant T and ET. It will also provide further data on dual HER2 suppression in an attempt to prevent acquired endocrine resistance.

Trial Design: Pts will be randomized to 1 of 3 treatment arms: L 1000 mg po QD plus T (loading dose of 8 mg/kg followed by maintenance with 6 mg/kg IV q3w plus an AI po QD); T plus an AI; or L 1500 mg po QD plus an AI. Choices of AI include letrozole, anastrozole, or exemestane.

Eligibility Criteria: HR+ (ER and/or PgR) and HER2+ Stage IV MBC pts are to be enrolled. Pts must have received neo-/adjuvant T and ET, and are treatment naive for MBC.

Specific Aims: The primary objective is to evaluate OS of L/T/AI as compared with T/AI. The secondary objectives are to assess: OS in T/AI vs L/AI and T/L/AI vs L/AI; PFS; overall response rate; clinical benefit rate; safety and tolerability; and QoL relative to baseline.

A 4-year recruitment is anticipated. More than 200 centers across 25 countries are planned; approximately 50 centers are currently open for enrollment.

Statistical Methods: The study is powered to detect a 42% improvement in the risk of death (HR=0.70) in all pts receiving L/T/AI (median 28.5 months) compared with T/AI (median 20 months). The hypothesis will be tested using a 1-sided test for superiority with \( \alpha = 0.025 \) with a power of 80%.

Present and Target Accrual: One (1) of 525 pts has been randomized. The majority of eligible pts may reside in countries where T is commercially available and reimbursable, particularly North America and Western Europe. Patients who have participated in previous neo-/adjuvant trials including a T regimen are eligible, provided they meet all other inclusion criteria.

The study is currently recruiting pts, with an anticipated target accrual of 525 patients by March 2016.

OT1-02-04

Adjuvant Pertuzumab and Herceptin In Initial Therapy of Breast Cancer: APHINITY (BIG 4-11/BO25126/TOC4939g).

von Minckwitz G, Baselga J, Bradbury I, de Azambuja E, Scullion MJ, Ross G, Saini KS, Piccart-Gebhart M. German Breast Group, Neu-Isenburg, Germany; Massachusetts General Hospital, Boston; Frontier Science and Technology Research Foundation, Scotland; BrEAST Data Center, Jules Bordet Institute, Brussels, Belgium; Roche, Welwyn Garden City, United Kingdom; Breast International Group Headquarters, Brussels, Belgium; Breast International Group, Brussels, Belgium

Background: Approximately 20% of breast cancer (BC) patients (pts) have HER2-positive tumors. While the adjuvant use of the anti-HER2 humanized monoclonal antibody trastuzumab (T) has been shown to improve disease-free (DFS) and overall survival (OS), not all pts treated with this agent benefit from this therapy. Pertuzumab (P) is a humanized monoclonal antibody that inhibits HER2 dimerization and induces ADCC with a complementary mechanism of action to T. In HER2-positive adjuvant BC, T and P are active in pts who have progressed to T. In the neoadjuvant setting, T and P in combination with chemotherapy (CT) nearly doubled the pathological complete response rate compared to either T or P administered in combination with CT (45.8% vs 29% vs 24%, respectively). Therefore, comprehensive HER2 blockade with two anti-HER2 monoclonal antibodies warrants further investigation in the adjuvant setting.

Trial Design: APHINITY is a prospective, randomized, multicenter, double-blind, placebo-controlled study in pts with HER2-positive primary BC who have had an excision of their tumor. Pts will be randomized to one of 2 arms (1:1 ratio). The investigational arm will comprise of a course of adjuvant CT (investigators choice) consisting of either an anthracycline-taxane or taxane-platin containing regimen and T and P for 1 year. The comparator arm will consist of the same adjuvant CT backbone with T and placebo for 1 year.

Major Eligibility Criteria:
1. Non-metastatic primary BC histologically confirmed and adequately excised
2. Node-positive or node-negative: for patients with node-positive disease (pN ≥1), any pT except T0; for patients with node-negative disease (pN0), tumor size must be >1.0 cm OR for tumor size between >0.5 cm and ≤1.0 cm, at least one of the following features will be required: histologic grade 3 OR negative for ER and PgR OR age ≥35 years.
3. The interval between definitive surgery for BC and randomization must be at least 3 weeks but no more than 7 weeks.
4. Baseline LVEF ≥55%
5. HER2-positive BC confirmed by a central laboratory and defined as: IHC 3+ in >10% immunoreactive cells OR HER2 gene amplification by in situ hybridization [ISH] (ratio of HER2 gene signals to centromere 17 signals ≥2).

Aims: The primary objective is to compare invasive disease-free survival (IDFS) between both treatment arms. Secondary objectives include comparing IDFS including second non-BC, DFS, OS, recurrence-free interval (RFI), distant RFI, cardiac safety, overall safety and health-related quality of life in the two treatment arms.

Statistical Methods: Pts will be stratified based on nodal status, type of adjuvant CT regimen, hormone receptor status and geographical region. The study is designed to have an 80% power to test the null hypothesis of no true difference in risk of an IDFS event (HR = 1) versus the alternative hypothesis of a difference (HR = 0.75) in hazard rates with a 5%, 2-sided significance level.

Target accrual: 3806; Present accrual: Start Q4 2011
OTI-02-05
A Phase III Clinical Trial Comparing Trastuzumab Given Concurrently with Radiation Therapy to Radiation Therapy (RT) Alone for Women with HER2-Positive DCIS Resected by Lumpectomy: NSABP B-43,
Julian TB, Anderson SJ, Cobleigh MA, Sziopikou KP, Arthur DW, Zheng P, Mamounas EP, Pajon ER, Behrens RJ, Chu L, Leasure NC, Atkins JN, Poliakov J, Seay TE, McCaskill-Stevens W, Rabinovitch R, Wolmark N. National Surgical Breast & Bowel Project (NSABP); Pittsburgh, PA; Allegheny General Hospital, Pittsburgh, PA; University of Pittsburgh Graduate School of Public Health and Pittsburgh, PA; Allegheny General Hospital, Pittsburgh, PA; Northwestern University Feinberg School of Medicine, Chicago, IL; Virginia Commonwealth University, Richmond, VA; Aultman Health Foundation, Canton, OH; Colorado Cancer Research Program, Denver, CO; Iowa Oncology Research Association, Des Moines, IA; Florida Cancer Specialists, Sarasota, FL; Reading Regional Cancer Center, West Reading, PA; SCCCC-CCOP, Goldsboro, NC; Kaiser Permanente Southern California, San Diego, CA; Atlanta Regional Community Clinical Oncology Program, Atlanta, GA; National Cancer Institute, Rockville, MD; University of Colorado, Aurora, CO

Background: Because a substantial portion of DCIS is ER negative and overexpresses HER2, therapy targeting this protein is a promising strategy for HER2-overexpressing DCIS. Preclinical studies have shown that trastuzumab (T) boosts the effectiveness of RT in xenograft models and in cell lines with no detrimental effect on irradiated HER2-normal cells. Studies correlating clinical response with molecular markers in T-treated patients show that apoptosis occurs within 1 wk of starting single-agent T, with little effect on proliferation. Shorter duration treatments with this agent require investigation. Adjuvant trials using T during breast irradiation have already provided ample safety evidence. Will T administered during WBI improve lumpectomy + WBI results in women with HER2-positive DCIS? This trial will allow us to better understand the biology of breast cancer and its prevention and will extend the benefits of breast-conserving surgery for women with DCIS.

Trial Design: Post lumpectomy for DCIS without evidence of an invasive component, a central review of each patient’s pure DCIS is carried out for HER2 by IHC analysis. If the HER2 is 2+ or FISH analysis is done, and patients whose tumors are HER2 3+ or FISH positive can be randomly assigned to receive 2 doses of T 3 wk apart during WBI or to receive WBI alone.

Eligibility criteria: Women 18 years or older with an ECOG status of 0 or 1 who have undergone a margin-clear lumpectomy for DCIS and whose tumors are clinically or pathologically node negative are eligible. DCIS must be HER2 positive by central testing. ER and HER2 PR status must be known before random assignment.

Specific aims: The primary aim is to determine if T given concurrently with WBI is more beneficial in preventing IBC recurrence, ipsilateral skin cancer recurrence, or ipsilateral DCIS recurrence compared with WBI alone for HER2-positive DCIS resected by lumpectomy. Secondary aims are to compare the possible benefit of T given during WBI to that of WBI alone in preventing regional or distant recurrence and contralateral invasive or DCIS breast cancer. B-43 will determine if invasive or DCIS DFS, recurrence-free interval, and OS can be improved with the addition of T to WBI. The effects of T on ovarian function in premenopausal women will also be assessed.

Statistical methods and accrual: Our design calls for accrual of 2000 patients during a 7.9-year period. As of May 31, 2011, 578 patients have been entered. A definitive analysis of primary endpoints will be performed when 163 ipsilateral breast cancer events occur (7.5 and 8 years after protocol initiation). This number of events affords 80% power to detect a hazard reduction of 36%, from 1.73 ipsilateral breast cancer events per 100 patient-years to 1.11 events per 100 patient-years. The 36% observed reduction in the hazard of IBTR-SCR-DCIS on the T arm is based on a projection of 40% hazard reduction if the compliance were perfect, with a 10% noncompliance rate.

Supported by PHS grants NCI-U10-CA-69651, NCI-U10-CA-12027, and NCI P30-CA-14599 from the US NCI and Genentech, Inc.

OTI-02-06
DETECT III – A Multicenter, Randomized, Phase III Study To Compare Standard Therapy Alone Versus Standard Therapy Plus Lapatinib in Patients with Initially HER2-Negative Metastatic Breast Cancer and HER2-Positive Circulating Tumor Cells.
Melcher CA, Janni W, Ortmann U, Jäger B, Rack B, Müller V, Schneweiss A, Pantel K, Solomayer E-F, Fehm T. University Hospital Duesseldorf, Duesseldorf, Germany; University Hospital Munich, Munich, Germany; National Center for Tumor Diseases Heidelberg, Heidelberg, Germany; University Hospital Hamburg, Hamburg, Germany; University Hospital Hamburg, Hamburg/Saar, Germany; University Hospital Tuebingen, Tuebingen, Germany; University Hospital Hamburg-Eppendorf, Hamburg-Eppendorf, Germany

Background
In breast cancer patients, HER2 status may change over the course of the disease. Approximately 20-30% of initially HER2-negative patients have HER2-positive metastasis (Zidan et al. 2005, Tewes et al. 2009). Re-evaluation of HER2 status on metastatic tissue is warranted, but not always possible, especially during the course of therapy. Determining HER2 status on circulating tumor cells is one option for re-evaluating HER2 status at the time metastasis is diagnosed as described in our previous study DETECT I (Fehm et al. 2010). However, at present it is unclear if therapy based on the HER2 status of CTC offers a clinical benefit for patients. Therefore, the study DETECT III aims to assess whether lapatinib, as one of the HER2-targeted therapies, in initially HER2-negative breast cancer patients with HER2-positive CTC is effective at the time of distant disease.

Trial Design
DETECT III is a prospective, multicenter, randomized, open-label, two arm phase III study. As only half of the patients with HER2-negative MBC will be CTC-positive and approx. 32% will exhibit HER2-positive CTCs a screening of about 1420 patients will be needed. Approx. 228 patients will be enrolled in the study and randomized 1:1 to one of the following regimens Arm A (n=114): Standard Treatment, Arm B (n=114): Standard Treatment plus Lapatinib.

Planned duration of individual study participation
• Max. duration of randomized treatment period: 12 months
• Max. follow-up period: 24 months

Main eligibility criteria
1. Metastatic breast cancer
2. HER2-negative primary tumor tissue and/or biopsies from metastatic sites or locoregional recurrences
3. Evidence of HER2-positive CTCs
4. Indication for a standard chemotherapeutic regimen with lapatinib is either approved or has been investigated in prior clinical trials
5. Tumor evaluation within 6 weeks before randomization
6. ≥ 1 lesion that can be accurately measured according to RECIST
7. LVEF ≥ 50

www.aacrjournals.org Cancer Res; 71(24 Suppl.) December 15, 2011
Specific aims
Objective
The objective of the trial is to prove the clinical efficacy of lapatinib in patients with metastasizing breast cancer who exhibit HER2-positive circulating tumor cells (CTC) although the primary tumor tissue and/or biopsies from metastatic sites were investigated for HER2 status and showed HER2-negativity.

Primary endpoint:
• Progression free survival
Secondary endpoints:
• Overall response rate
• Clinical benefit rate
• Overall survival
• Dynamic of CTC
• Quality of life
• Safety and tolerability of lapatinib

Statistical methods
The primary endpoint will be analyzed by Kaplan-Meier method using the logrank test in order to compare the PFS distributions of the two arms. Efficacy, toxicity and other event rates are calculated, providing confidence intervals. In case of comparison between patient groups, these rates will be analyzed by Fisher’s exact test or χ² test. The Kaplan Meier analysis for all event related data will be carried out overall for the whole patient population. Furthermore a Cox regression analysis will be done using the following covariates
• Hormone receptor status (positive/negative)
• Number of prior chemotherapy lines for metastatic disease
• Prior endocrine therapy for metastatic disease
• Endocrine treatment vs. cytotoxic treatment
• One metastatic site vs. multiple metastatic sites
• Bone metastases vs. no bone involvement
• Performance status (0 / > 0)

OT1-02-07

Fehrenbacher L, Jeong J-H, Rastogi P, Geyer CE, Paik S, Ganz PA, Land SR, Costantino JP, Swain SM, Mamounas EP, Wolmark N. National Surgical Adjuvant Breast and Bowel Project (NSABP) Operations and Biostatistical Centers; Kaiser Permanente, Northern California; University of Pittsburgh Graduate School of Public Health; University of Pittsburgh Cancer Institute, University of Pittsburgh School of Medicine; Allegheny General Hospital; Jonsson Comprehensive Cancer Center at UCLA; Washington Cancer Institute, Washington Hospital Center; Aultman Health Foundation

Adjuvant studies utilizing trastuzumab in early HER2+ breast cancer demonstrated a large reduction in recurrence and death. Post-enrollment central testing showed HER2 non-amplified participants derived similar benefit. Among HER2-amplified patients, multiple studies showed no effect on benefit by degree of amplification. Extensive testing including blinded external review confirmed the non-amplified nature of the HER2 normal group. Detailed relevant background and confirmatory studies will be provided. As a result of these findings, NSABP study B-47, sponsored by the NCI, was activated January 2011. The study is NCI central IRB approved, open in the CTSU, and endorsed by SWOG as of April 2011.

Study: Selection of one of the two chemotherapy regimens is by physician choice: The non-anthracycline regimen is TC (docetaxel 75 mg/m2, cyclophosphamide 600 mg/m2) administered IV every 3 weeks for 6 cycles; the anthracycline regimen is AC followed by WP (doxorubicin 60 mg/m2 and cyclophosphamide 600 mg/m2 administered IV either every 3 weeks or every 2 weeks [per investigator discretion] for 4 cycles followed by paclitaxel 80 mg/m2 IV weekly for 12 doses). Patients will be randomly assigned to receive chemotherapy with or without trastuzumab therapy. For patients receiving the TC chemotherapy regimen, trastuzumab will be given every 3 weeks during and following chemotherapy until 1 year after the first trastuzumab dose (8 mg/kg loading dose; 6 mg/kg for the remaining doses). For patients receiving the AC followed by WP chemotherapy regimen, trastuzumab will begin with the first dose of weekly paclitaxel and will be given weekly for 12 doses (4 mg/kg loading dose; 2 mg/kg for the remaining weekly doses). Following completion of WP, trastuzumab therapy will continue with 6 mg/kg doses given every 3 weeks for a total of 1 year. Patients will also receive adjuvant radiation therapy and endocrine therapy, as clinically indicated.

Detailed menstrual history, concurrent medications, weight changes, and biomarkers (estrogen, stress, inflammation status) will be collected throughout the study. Collection of circulating tumor cells as an ancillary study is planned.

Eligibility: Eligibility includes: node positive or high risk node negative female breast cancer patients; HER2 IHC 1+ or 2+ scores, but non amplified by FISH; normal cardiac, renal, and liver function. Detailed eligibility will be provided.

Statistical: The primary aim is to determine whether the addition of trastuzumab to chemotherapy improves invasive disease-free survival (IDFS).

3260 patients will be enrolled to provide statistical power of 0.9 to detect a 33% reduction in the hazard rate of IDFS using a one-sided alpha level of 0.025. Projected accrual time is approximately 3 years.

Progress: Protocol was activated in January 2011. First patient was entered in February 2011. As of June 16, 2011, 115 of 3260 patients have been enrolled. Supported by NCI U10-12027, -37377, 69651, 69974, and Genentech, Inc.

OT1-02-08
The PERSEPHONE Trial: Duration of Trastuzumab with Chemotherapy in Women with HER2 Positive Early Breast Cancer. Changing the Randomisation Point To Address Potential Barriers to Recruitment.

Earl HM, Lai S, Vallier A-L, Hiller L, Ogburn-Storey E, Higgins H, Dunn J. Addenbrooke’s Hospital, Cambridge, United Kingdom; University of Warwick, Coventry, United Kingdom

Background: Persephone is a phase III randomised controlled trial comparing six months of trastuzumab (9 doses) to the standard 12 month duration (18 doses) in patients with HER2 positive early breast cancer in respect of disease free survival, safety and cost-effectiveness. The trial opened to recruitment in October 2007 but soon showed that meeting recruitment targets was challenging, reaching only 20-30 patients per month. A key issue seemed to be the mandatory requirement to randomise before patients started trastuzumab treatment. Successful accrual of patients in the PHARE trial, a Persephone sister study, run by the National Institute for Cancer, Paris had not incorporated this criteria.

Method: In September 2009, following accrual of 316 patients, this potential barrier to recruitment was addressed by a major
protocol amendment which relaxed the eligibility criteria to allow randomisation of patients who had received up to 9 doses of trastuzumab.

Results: To date, 1334 patients have been randomised into PERSEPHONE. After the amendment, monthly recruitment increased to 40-50 patients and, more recently, to 60-70 patients. Of the 1018 patients recruited since September 2009, 450 (44%) patients had received at least 1 dose of trastuzumab pre-randomisation (see Table).

<table>
<thead>
<tr>
<th>Number of trastuzumab doses received</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>557</td>
<td>55%</td>
</tr>
<tr>
<td>1</td>
<td>142</td>
<td>14%</td>
</tr>
<tr>
<td>2</td>
<td>74</td>
<td>7%</td>
</tr>
<tr>
<td>4</td>
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<td>4%</td>
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<td>5</td>
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<tr>
<td>6</td>
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<td>7</td>
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<td>9</td>
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<tr>
<td>11</td>
<td>1</td>
<td>1%</td>
</tr>
<tr>
<td>Unknown</td>
<td>11</td>
<td>1%</td>
</tr>
</tbody>
</table>

Retrospective collection of pre-randomisation trastuzumab dose and toxicity information has proved successful, allowing analyses of dose intensity, toxicity and compliance to be carried out on all patients.

Conclusion: Relaxing the eligibility criteria has considerably improved recruitment into the PERSEPHONE trial without compromising the important endpoints the trial sets out to assess.

OTI-02-09
A Phase II Randomized Trial of Lapatinib with Either Vinorelbine or Capecitabine as First- and Second-Line Therapy for HER2-Overexpressing Metastatic Breast Cancer.

Janni W, Pikiel J, Sarosiek T, Karaszewska B, Papadimitriou CA, Schwedler K, Alavarez Gallego J, Caruso M, Herve RA, Lau MR, Williams LS, Briggs K, Sapunar FJ, Heinrich-Heine-Universität; Wojewódzkie Centrum Onkологии; Centrum Medyczne Ostrobramska; Przychodnia Lekarska KOMED; Alexandria Hospital; Johann-Wolfgang-Goethe-Universität; Hospital Provincial de Zama; Humanitas Centro Catanese di Oncologia; Centre Medical Claval; GlaxoSmithKline

Background: Lapatinib, a dual kinase inhibitor of epidermal growth factor receptor (EGFR) and the human epidermal growth factor receptor-2 (HER2/ErbB2), is approved for the treatment of HER2-overexpressing (HER2+) metastatic breast cancer (MBC) in combination with capecitabine following progression after trastuzumab, anthracyclines, and taxanes. Vinorelbine is an important chemotherapy option in MBC, and multiple Phase II trials in combination with trastuzumab have been conducted.

Methods: This randomized, open-label, multicenter, phase II study (VITAL, LAP112620, NCT01013740) is evaluating the efficacy and safety of lapatinib with either vinorelbine or capecitabine in women with HER2+ MBC. A total of 105 stage IV breast cancer patients with disease progression who have received ≤1 chemotherapy regimens in the metastatic setting with an ECOG performance status of ≤1 are randomized 2:1 to either: lapatinib 1250 mg orally once daily (QD) continuously plus vinorelbine 20 mg/m² intravenously on days 1 and 8 every third week; or lapatinib 1250 mg orally QD continuously plus capecitabine 2000 mg/m²/d orally in 2 doses 12 hours apart on days 1 to 14 every third week. Following progression in the randomized phase, patients will be given the option to cross over to the other arm. The primary endpoint is progression-free survival and will be analyzed with a descriptive intent since the study is not powered to detect differences between treatment arms. Secondary endpoints include overall response rate, overall survival, duration of response, time to response, and clinical benefit rate.

The study is currently recruiting in 8 countries in Europe (Bulgaria, France, Germany, Greece, Italy, Poland, Serbia, Spain) and 2 in Latin America (Chile, Mexico).

Funding Source: GlaxoSmithKline

OTI-02-10
Phase I-II Study of HER2 Vaccination with Poly(I) • Poly(C₃U) (Ampligen®) as an Adjuvant in Optimally Treated Breast Cancer Patients.

Higgins DM, Childs J, Parker S, Guthrie KA, Disis ML, Salazar LG. University of Washington, Seattle, WA; Fred Hutchinson Cancer Research Center, Seattle, WA

Background: Despite improved response rates and overall survival, many HER2+ breast cancer (BC) patients have disease relapse suggesting residual microscopic disease. HER2 vaccines given with adjuvants that can enhance, sustain, and skew antigen immunogenicity toward a Th1 phenotype could induce robust tumor-specific Th1 immunity resulting in immunologic eradication of residual tumor cells and potentially prevent relapse. One such adjuvant is Ampligen which is highly selective as a TLR-3 agonist. Our pre-clinical studies show a dose effect in the tumor prevention efficacy of Ampligen when given as an adjuvant with vaccines. We hypothesize HER2 peptide vaccination given with standard adjuvant 100mcg GMCSF and Ampligen can induce a higher incidence and magnitude of protective HER2-specific Th1 immunity than with GMCSF alone.

Trial design: Phase I-II randomized 2-stage HER2 vaccine study. Stage I will enroll 40 patients (10/arm) into one of 4 Ampligen dose arms (4, 20, 79, or 495 mcg + HER2 vaccine). The Ampligen “maximum biologic dose” (MBD), the dose with the highest incidence/magnitude of immune response and lowest incidence of toxicity will be defined. Stage II will enroll 48 patients (24/arm) receiving Ampligen MBD + HER2 vaccine + GMCSF or HER2 vaccine + GMCSF to evaluate if Ampligen MBD increases the incidence and magnitude of immunity vs HER2 vaccine + GMCSF alone. Patients will be enrolled sequentially and randomized equally into all arms via a permuted block design. Patients will receive 3 monthly vaccines. Toxicity and immune response will be assessed.

Aims: 1) To evaluate toxicity and define the MBD of Ampligen as an adjuvant with HER2 vaccination 2) determine if Ampligen MBD when combined with GMCSF as adjuvant and HER2 vaccination increases incidence/magnitude of HER2 Th1 immunity compared to standard GMCSF alone.

Eligibility criteria: Stage II--IV HER2+ BC patients who: 1) have completed definitive standard treatment, and in clinical remission 2) 14 days post chemotherapy and steroids 3) have adequate blood counts 4) are off trastuzumab 5) have no active autoimmune disease.

Statistical methods: In aim 1, we expect mild toxicity between the 4 dose arms, thus lack of efficacy based on incidence of immune response will be evaluated. Six responses must be observed within a dose arm to move forward based on historical 60% response rate (RR) with standard GMCSF (probability of continuing if true RR is 40% and 70% is 0.17, 0.85, respectively). In aim 2, 24 patients/arm provides 80% power to detect 40% difference in incidence of immune response between the 2 groups (Pearson chi-square test, two-sided alpha of 0.05) and 82% power to assess a 0.85 SD unit difference in change between control and MBD, based on a 2-sample t-test (p=0.05) and effect size defined as the difference in the means divided by the common SD. Incidences of HER2 Th1 immunity will be compared across treatment arms via Pearson chi-square test; magnitude of immune response will be compared across groups via linear regression model.
Study Accrual: Target accrual is 88 patients: Stage I (n=40) and Stage II (n=48). There has been no accrual at this time.

OT1-02-11
MotHER: A Registry Developed for Women with Breast Cancer Who Have Received Trastuzumab within 6 Months Prior to Conception or during Pregnancy.

Background: Trastuzumab is a humanized monoclonal antibody that targets HER2, a protein that is overexpressed in approximately 20% of patients with breast cancer (BC). Trastuzumab is approved for the treatment of HER2-positive BC in both the metastatic and adjuvant settings, and is classified as US Food and Drug Administration (FDA) Pregnancy Category D, which indicates evidence of fetal harm, but benefit may outweigh risk. In the postmarketing setting, oligohydramnios has been reported in patients who received trastuzumab during pregnancy either alone, or in combination with chemotherapy. As a result, this registry was requested by the FDA and was established as part of a post-marketing commitment.

Study Design: MotHER uses a prospective, observational cohort design to evaluate the outcomes of pregnancies in women exposed to trastuzumab within 6 months prior to conception or during pregnancy. Women are followed until pregnancy outcome is known; infants are followed through the first year of life. Medical information is primarily collected from the patient’s healthcare providers (HCPs) (ob/gyn, oncologist, pediatrician). Using a standardized recording and coding system, information collected on potential defects noted at birth or during the pediatric follow-up period is evaluated and classified by a birth defect evaluator/clinical geneticist.

Eligibility Criteria: Enrollment is voluntary and must be initiated by the patient before the pregnancy outcome. Enrollment may be suggested by an HCP. A verbal informed consent process is used. Patients with known prenatal testing results may enroll; however, to reduce the potential for bias, patients with knowledge of prenatal testing results (either normal or abnormal) will be analyzed as a separate subset.

Aims: The aim of this study is to evaluate the effects of trastuzumab therapy on pregnancy outcome in women who were administered trastuzumab while pregnant. The MotHER registry is the first prospective cohort study to be established for investigating the effects of a targeted cancer therapy on pregnancy outcome. The greatest challenge for this registry is to enroll a sufficient number of patients from the relatively small population of trastuzumab-exposed women who become pregnant.

Analysis Methods: Primary outcome measures are the occurrences of oligohydramnios, live births, fetal deaths/stillbirths (≥20 weeks gestation), and fetal/infant major malformations, deformations, disruptions or functional deficits. Secondary outcomes include pre-term births, elective terminations, miscarriages, fetal growth abnormalities, and pregnancy or delivery complications. The sample size has not been pre-specified. The study is descriptive: event proportions and associated confidence intervals will be calculated; statistical testing is inappropriate.

Accrual: This registry was activated in December 2008 and will accrue in the US over a 10-year period. As of June 20, 2011, 5 patients had been enrolled.

Contact Information: Patient and HCP awareness of the registry and willingness to participate are crucial to its ultimate success.

OT1-02-12
Early Detection of Cardiotoxicity by Advanced Cardiac Imaging and a Novel Biomarker in Breast Cancer Patients Undergoing Chemotherapy.

Background
The survival rate of breast cancer patients has increased due to improvements in cancer treatment. However, many survivors develop irreversible or reversible cardiotoxicity associated with anthracycline or trastuzumab therapy, respectively. To detect cardiac damage, the currently accepted method is to measure left ventricular ejection fraction (LVEF) by echocardiography, which lacks the sensitivity to predict early cardiac dysfunction.

Early identification of cardiotoxicity is essential to cancer survivors, as development of cardiomyopathy carries a worse outcome independent of cancer prognosis. Currently, there are no accepted guidelines for the early detection of myocardial injury. The use of cardiac biomarkers and more sensitive echocardiographic techniques have expanded options for monitoring, but have yet to reach a consensus.

Hence, our study will evaluate the potential predictive value of novel cardiac biomarkers and advanced echocardiographic and cardiac magnetic resonance imaging (CMR) techniques to detect subclinical myocardial damage. Our findings may be applicable for monitoring new antineoplastic agents during food and drug administration (FDA) clinical trials.

Trial Design
Prospective cohort study with internal control of 20 patients newly diagnosed with breast cancer. The trial will assess endpoints at baseline, 2 weeks after initiation of therapy, and 2 weeks and 6 months after chemotherapy completion.

1. Primary Endpoint
   a. Decline in left ventricular ejection fraction assessed by CMR and 3D-echo not detected by conventional methods
   b. Presence of either myocardial fibrosis or edema detected by CMR
   c. Changes in myocardial deformation detected by echo or CMR strain
   d. Increase in cardiac biomarkers (Serum caspase-3 p17 peptide, Troponin I, B-type natriuretic peptide) and possible correlation with imaging parameters

2. Secondary Endpoint
   a. Development New York Heart Association class 1 to 4 symptoms
   b. Decrease in LVEF of ≥5% to ≤50% with or without symptoms

Eligibility Criteria
Inclusion Criteria:
1. Newly diagnosed stage I, II, or III breast cancer
2. Age between 18 and 75 years old.
3. Treatment with trastuzumab or anthracycline-based chemotherapy

Exclusion Criteria:
1. History of cardiovascular disease
2. Pacemaker
3. History of mediastinal radiotherapy
4. Creatinine clearance <30 ml/min
5. Serum bilirubin >2.0 mg/dl, ALT and AST > 100 U/l
6. Hypertension, uncontrolled >140/90
7. LVEF <55% per 2-D echocardiogram
8. Claustrophobia

Specific Aims
1. Detect early myocardial injury.
2. Evaluate early predictors of left ventricular dysfunction.
3. Evaluate timing of monitoring during or post treatment
Statistical Method
This is a pilot study and 20 patients are required to reach statistical significance with 85% power. All values will be analyzed as mean±SD or n (%). Categorical indicators will be analyzed using nonparametric statistics such as Cochran’s Q. Changes in imaging and biomarker parameters will be assessed using analysis of variance, while correlation between the two will be assessed using mixed models appropriate for binary outcome. Significance will be accepted at p ≤0.05 for all tests.

Present accrual and target accrual
Nine subjects are enrolled with a goal of 20.

Contact for people interested in trial:
1. Dr. Erick Avelar, eavelar@uchc.edu
2. Dr. Susan Tannenbaum, stannenbaum@uchc.edu

OT1-02-13
Cardiac Biomarkers on Trastuzumab (Cabot Trial): Determining the Cardiac Biomarker Profile in Breast Cancer Patients Receiving Adjuvant Trastuzumab Therapy.
Kumar VNC, Kavask P, Rask S, Mukherjee SD, Ellis P; Dhesy-Thind B. Sunnybrook Odette Cancer Centre, Toronto, ON, Canada; Juravinski Hospital, Hamilton, ON, Canada; Juravinski Cancer Centre, Hamilton, ON, Canada

Background: Trastuzumab is a humanized anti-HER2 monoclonal antibody that has demonstrated a 50% reduction in the risk of HER2+ breast cancer recurrence. This benefit is at the risk of possible cardiac dysfunction. Detecting trastuzumab-related cardiotoxicity based on physical examination and assessment of left ventricular ejection fraction (LVEF) by serial echocardiography or multi gated acquisition (MUGA) scan, has low diagnostic sensitivity and low predictive power in detecting subclinical myocardial injury. There is interest in developing simple, non-invasive, and cost-effective tools for early identification of trastuzumab-related cardiotoxicity. Use of easily detectable cardiac biomarkers in blood, such as cardiac troponins (cTnT and cTnI) and natriuretic peptides (NPs), are being evaluated. Kavask et al. have studied a high-sensitivity cTn (hs-cTn) assay that is 10-fold more sensitive, and may be able to identify evolving injury earlier when compared to the conventional cTn assays. B-type natriuretic peptide (BNP) has been established as an indicator for heart failure. Pro-BNP is secreted from the cardiac ventricles in response to increased pressure and volume and is divided into NT-pro-BNP and BNP. The longer half-life of NT-proBNP may make it a more accurate predictor of ventricular stress. Trial design: This is a pilot single institution, prospective cohort study of 25 patients. Breast cancer patients will be seen at the Juravinski Cancer Centre, Hamilton, Canada. Biomarkers will be collected at baseline, then days 1 and 2 of every 21 day cycle for the first 3 months, then every 42 days for the next 3 months. LVEF will be measured at baseline and then every 3 months while on trastuzumab. Eligibility criteria: Patients with stage I-III, HER2 positive (3+ by immunohistochemistry or FISH+) breast cancer receiving adjuvant trastuzumab therapy. Patients who have received prior trastuzumab therapy or are unable to provide informed consent will be excluded. Specific aims: To determine the biological profile of cardiac biomarkers – cTnT, hs-cTn, and NT-proBNP – in patients on adjuvant trastuzumab; and, to determine if elevations in these biomarkers correlate with LVEF. Statistical methods: A convenient sample size of 25 patients will be entered into this pilot study. The minimum of 20 patients is a requirement by the Clinical Laboratory Standards Institute (CLSI) to formally accept existing reference ranges for the cardiac biomarkers to be used in the HER2 breast cancer population (National Committee for Clinical Laboratory Standards (NCCLS)). Present accrual and target accrual: 8 patients have enrolled to date.

OT1-03-01
A Randomized Phase III Clinical Trial of Standard Adjuvant Endocrine Therapy +/- Chemotherapy in Patients (pts) with 1-3 Positive Nodes, Hormone Receptor (HR)-Positive and HER2-Negative Breast Cancer with Recurrence Score (RS) of 25 or Less: SWOG S1007.
Gonzalez-Angulo AM, Barlow WE, Gralow JR, Meric-Bernstam F, Hayes DF, Moinpour CM, Ramsey SD, Schott AF, Sparks DB, Albain KS, Hortobagyi GN. MD Anderson Cancer Center; Houston, TX; Cancer Research And Biostatistics, Seattle, WA; Seattle Cancer Care Alliance/University of Washington, Seattle, WA; University of Michigan, Ann Arbor, MI; Fred Hutchinson Cancer Research Ctr, Seattle, WA; University of Michigan and SWOG, Ann Arbor, MI; SWOG, San Antonio, TX; Loyola Univ Chicago Stritch School of Medicine, Maywood, IL

Background: Multi-gene tumor assays have provided clinically useful prognostic information for pts with HR receptor and node-positive breast cancer. The 21-gene RS was shown to be prognostic for pts treated with tamoxifen alone, and exploratory studies suggested that it may be predictive of benefit from chemotherapy. In retrospective analyses from SWOG S8814, pts with low RS appeared to get no benefit from adjuvantCAF chemotherapy, while those with higher RS did. These retrospective data require validation, especially since more modern chemotherapy might be more effective than the regimen used in S8814.

Specific Aims/Trial Design: In January 2011, SWOG activated a phase III randomized clinical trial (registration number NCT01272037) to test the efficacy of using modern chemotherapy regimens in node positive pts with low RS, whose prognosis is still moderately poor but may not benefit from adjuvant chemotherapy based on tumor biology predicted by the RS value. The trial is similar to the Tailor RX study, but focuses on a node-positive population with low and intermediate RS.

Eligibility Criteria: Pts with 1 to 3 positive lymph nodes, HR-positive and HER2-negative invasive breast cancer with RS ≤ 25 are eligible for randomization. Pts will be informed of their RS, Target and Present Accrual: Approximately 9400 patients will be screened to randomize 4000, stratified by RS (0-13 vs. 14-25), menopausal status, and axillary surgery (sentinel node vs. complete dissection); 46 are presently registered. Statistical Methods: The trial is powered to find a significant interaction of treatment assignment and the continuous RS value and, subsequently, derive a cut point for using the assay to guide treatment decisions. Pts who consent to screening are required to consent to banking of the tumor tissue and blood for further studies. Patient Reported Outcomes will be collected pre, post-screening and post-randomization. The study also has a cost-effectiveness analysis. Funding: Supported in part by National Cancer Institute grants CA32102 & CA38926, and in part by the Susan G. Komen for the Cure® Research Program, and the Breast Cancer Research Foundation.
OT1-03-02
SAFIR01: A Molecular Screening Trial for Metastatic Breast Cancer Patients.
Andre F, Peletkian C, Jimenez M, Ferrero JM, Delaloge S, Roman Roman S, Dessen P, Bonnefoi H. Gustave Roussy Institute; Unicancer; Curie Institute; Bergonie Institute; Centre antoine lacassagne, Nice, France

Background: High number of new drugs are targeting molecular alterations that occur in a few number of patients. Molecular screening that consists in the assessment of single molecular alteration during the screening phase of the therapeutic trial is not suitable. Indeed, this modality of screening is associated with a high rate of screen failure due to the low incidence of the molecular alteration. In order to optimize molecular screening, we have launched a clinical trial that aims at performing array CGH and hot spot mutations in patients with metastatic breast cancer.

Patients and Methods. The trial plans to include 400 patients with metastatic breast cancer and included the first patient in may 2011. This trial is being sponsored by French federation of Cancer centers (UNICANCER) and involves 20 centers. Biopsy is being performed on metastatic site. Both frozen and FFPE samples are obtained. DNA extraction is being performed in the investigation center after control for the % of cancer cells. Array CGH and PIK3CA/AKT mutations (SANGE method) are being performed in four genomic platforms. A pilot study that included 106 patients has shown the feasibility of such technologies in the context of daily practice. Data from array CGH are being sent to a bioinformatician who forward the results on both pre-selected targets, DNA instability and some targets of interest selected based on log2(ratio) and function. Recommendations for trials are then being sent to each investigator. There is no limitation for previous lines and it is recommended to perform biopsy in patients who do not present progressive disease. This trial is being funded by French NCI (750 000 euros)

Conclusion: SAFIR01 is a trial that aims at using high throughput technologies in order to drive patients with molecular alterations to specific therapeutic trials. Further amendments are being planned including implementation of high throughput sequencing and performance of functional testing. Further trial will compare this high throughput approach to standard methods for target identification.

OT1-03-03
Effect of Tamoxifen Therapy on Inhibition of Tumor Suppressor p53 by Estrogen Receptor.
Kulkarni S, Fetterly GJ, Morrison CD, Adjei AA, Andrews C, Edge SP, Mukhopadhyay UK, Swezigg WM, Das GM. Roswell Park Cancer Institute, Buffalo, NY

Background: A large number of patients with estrogen receptor (ER)-positive tumors are resistant to tamoxifen (TAM). Although several plausible reasons for such resistance have been suggested, the mechanisms remain unclear. ER mediates effects of estrogen by promoting proliferation of breast cancer cells. Tumor suppressor protein p53 guards against tumorigenesis by preventing proliferation of cells with genomic damage. Dr. Das’s laboratory previously reported that ER binds and functionally suppresses wild type p53 in human breast cancer cells and xenograft tumor tissue, and TAM is capable of inhibiting this interaction. We hypothesize that relieving suppression of wild type p53 by ER could be an important mechanism underlying TAM action in breast cancer. To test this hypothesis, we have initiated a pilot randomized clinical trial of 50 women with newly diagnosed ERα-positive breast cancer.

Specific Aims: 1) To investigate the effect of a short pre-surgical intervention with TAM on the ER-p53 interaction in ER-positive, p53 wild type breast tumors and 2) To confirm the wild type status of p53 and analyze the functional status of the p53 pathway by monitoring expression of selected p53 target genes in tumors that have and have not been treated with TAM.

Trial design: A randomized clinical trial in the pre-surgical setting was proposed with either TAM 20mg for four weeks vs. no intervention. Patients randomized to the TAM arm will undergo multiple pharmacokinetic and pharmacodynamic measurements of TAM metabolites and genotyping for common polymorphisms of TAM metabolism genes, CYP2D6 and CYP3A4/5. Fresh tumor tissue will be harvested from all patients at the time of surgery for analysis of ER-p53 protein interaction using tissue chromatin immunoprecipitation (tissue ChIP) assay. p53 gene status will be determined by sequencing. RNA and protein expression of ER, p53, and a selected group of ER and p53 target genes in the diagnostic core biopsy and surgical specimens will be analyzed by quantitative real-time PCR (qRTPCR) and immunohistochemistry (IHC). TAM metabolites will also be measured in the tumor and the surrounding benign tissue.

Eligibility criteria: Women greater or equal to eighteen years of age diagnosed with ER-positive invasive breast cancer (approximately 1cm in size) who will undergo primary surgical excision for their initial therapy are eligible. Women must not be pregnant, be on current hormonal therapy, or have a history of hypercoagulable syndrome or prior arterial or venous thrombosis.

Statistical methods: Descriptive statistics such as frequencies and relative frequencies will be computed for all categorical variables. Numeric variables will be summarized using simple descriptive statistics such as mean, standard deviation, and quartiles. Ninety-five percent confidence intervals will be computed. ChIP data will be subjected to Fisher’s Exact Test. IHC date will be analyzed by Wilcoxon-Mann-Whitney test.

Present accrual and target accrual: We have accrued 17 patients to date and plan to accrue a total of 50 patients, 25 in each arm.

OT2-01-01
International Breast Cancer Study Group (IBCSG) Trial 22-00: Low-Dose Cytotoxics as Maintenance “Anti-Angiogenesis Treatment” Following Adjuvant Induction Chemotherapy for Patients with ER-Negative and PgR-Negative Breast Cancer.
Colleoni M. International Breast Cancer Study Group

Background
Patients with endocrine responsive disease benefit from five years of endocrine therapy after chemotherapy, but for patients with endocrine non-responsive disease there is no evidence of benefit of extending treatment beyond a standard duration (three to six months). The low-dose maintenance chemotherapy (metronomic cyclophosphamide and methotrexate (CM) given orally) regimen being tested in this trial was developed in advanced disease and resulted in tumor shrinkage even in heavily pretreated patients. The mechanism of this effect might be through anti-angiogenesis or by interference with progression of the stromal structure of metastases.

Trial Design
This randomized phase III trial compares 12 months of CM Maintenance chemotherapy (CMM) (cyclophosphamide 50/mg/day orally continuously for 1 year; methotrexate 2.5 mg/twice a day orally days 1 and 2 of every week for 1 year) vs. no CMM, following breast cancer surgery and standard induction chemotherapy. Stratification factors: institution, menopausal status, induction regimen. Concurrent trastuzumab is permitted for patients with HER2-positive disease.
Major Eligibility Criteria
-Histologically proven primary breast cancer
-Both estrogen and progesterone receptor status negative (<10% of tumor cells positive by IHC)
-Known HER2 status
-Approved induction chemotherapy must begin within 8 weeks after definitive surgery
-Pathological axillary staging
-

Specific Aim
To evaluate the efficacy of a low-dose chemotherapy regimen, hypothesized to have anti-angiogenic activity, administered following a standard chemotherapy program, in patients whose tumors are not endocrine therapy-responsive.

Statistical Methods
The primary analysis population will be intention-to-treat (all randomized patients). The primary endpoint, disease-free survival (DFS), will be compared between treatment arms using a two-sided stratified logrank test with an alpha level of 0.05. Based on results from ER-negative populations in other IBCSG trials, we assume that the overall five-year disease-free survival for the group receiving induction chemotherapy alone will be 70%. Due to a relatively high non-adherence rate (14% of early patients randomized to CMM did not start CM), the original sample size of 900 was increased to 1080 (October 2010), and the target number of events from 256 to 307. With this revision detection of a true hazard ratio of 0.70 will be achieved with a power of 72% despite a 15% non-adherence rate in the CMM group.

Present and Target Accrual
Target: 1080 patients. Present: 967 (as of June 1, 2011).

Related Research
Serum Substudy for 170 patients will evaluate differences from baseline to 18 months post baseline in serum VEGF, VCAM-1 and NRP protein values. Patient self-reported quality-of-life assessments are collected at baseline and months 9, 12, 18, 24 for all patients.

Central pathology review of histology, grade, ER, PgR, Her2, and Ki-67 for all patients is ongoing.

OT2-01-02
First-Line Bevacizumab in Combination with Capecitabine or Paclitaxel for HER2-Negative Locally Recurrent or Metastatic Breast Cancer (LR/MBC): A Randomized Phase III Trial.
Beslja S, Brodowicz T, Greil R, Inbar MJ, Kahán Z, Kaufman B, Lang I, Sieger GG, Stemmer SM, Zielinski C, Zvirbule Z, The CECOG TURANDOT Trialists. Institute of Oncology, Sarajevo, Bosnia and Herzegovina; Medical University of Vienna, Vienna, Austria; University Hospital Salzburg, Salzburg, Austria; Tel Aviv Sourasky Medical Centre, Tel Aviv, Israel; University of Szeged, Szeged, Hungary; Sheba Medical Center, Tel Hashomer, Israel; National Institute of Oncology, Budapest, Hungary; Rabin Medical Center, Petah Tikva, Israel; Riga Eastern Clinical University Hospital, Riga, Latvia

Background: A number of phase III studies have shown significant progression-free survival (PFS) benefits with the combination of bevacizumab (Bev) and either first-line capecitabine (X) or taxane therapy in LR/MBC. The ongoing open-label, randomized, phase III CECOG-sponsored TURANDOT study (CECOG/BC.1.3.005) is investigating the efficacy and safety of first-line Bev plus paclitaxel (P) versus Bev plus X in this setting.

Materials and methods: Eligible patients (pts) are aged ≥18 years with HER2-negative, chemonaive LR/MBC, an EOCG performance status of 0–2 and a life expectancy >12 weeks. Prior chemotherapy and concomitant hormonal therapy for LR/MBC are not permitted, but prior (neo)adjuvant chemotherapy is allowed if completed ≥6 months before randomization or ≥12 months if taxane based. Pts are randomized to receive Bev 10mg/kg days 1, 15 plus P90mg/m² days 1, 8, 15, q28d (Arm A) or Bev 15mg/kg day 1 plus X 1,000mg/m² bid days 1–14, q21d (Arm B) until disease progression, unacceptable toxicity or withdrawal of consent. The primary objective is to demonstrate non-inferiority in overall survival (OS) with Bev plus P versus Bev plus X (upper limit ≤1.33 for the two-sided confidence interval for hazard ratio [HR]). Secondary objectives are: comparison of overall response rate (RECIST criteria); PFS; time to response; duration of response; time to treatment failure; quality (CTCAE version 3); and quality of life (EORTC QLQ-30). The recruitment target is 560 pts. A sample size of 490 pts in the per-protocol population will be required to provide 80% power to reject the null hypothesis of inferiority at a one-sided significance level of 0.025, assuming a 24-month median OS with Bev plus P and an alternative hypothesis of HR=1. Data cut-off for adverse event reports was 12 Apr 2010. Interim and final efficacy analyses will be triggered after 175 and 389 events, respectively.

Results: Recruitment to the study began in Sep 2008 and was completed in Aug 2010, with 561 pts randomized. Follow-up is ongoing.

Conclusions: TURANDOT is the first study to examine the efficacy and safety of Bev plus P versus Bev plus X as first-line treatment for pts with LR/MBC.
≥4 cycles of anthracycline and/or taxane containing neoadjuvant chemotherapy regimen (+trastuzumab in HER2-positive tumors) are eligible. Pts must have failed to achieve a pCR following neoadjuvant treatment (i.e. residual invasive breast cancer >5mm) in the breast or lymph nodes at surgery [ypT1b-T4, N1-2, M0]). Additional eligibility criteria include: ECOG performance status of 0-1; adequate hematologic, hepatic and renal function. Pts with nonhealed surgical wounds, known or active cardiovascular disease, QTC interval > 480 ms, chronic use of QTc prolonging drugs, as well as previous breast cancer diagnosis <3 years prior to trial entry, are excluded.

**Trial Design:** Pts are stratified into three cohorts according to HER2 and hormone receptor status as follows: triple-negative (Cohort A, n=54), hormone-receptor-positive/HER2-negative (Cohort B, n=42) and HER2-positive (Cohort C, n=32). All pts receive eribulin mesylate 1.4mg/m² IV on days 1 & 8 of each 21–day treatment cycle for 6 cycles. Pts with HER2-positive tumors also receive trastuzumab 6mg/kg IV day 1 of each cycle. Locoregional radiotherapy and/or adjuvant hormonal therapy (Cohort B only) will be administered per institutional guidelines. With standard neoadjuvant therapy, the approximate median DFS of pts who do not achieve 2-pCR are as follows: triple-negative, 40%; hormone-receptor-positive/HER2-negative, 80%; and HER2-positive, 60%. For a one-sided test of hypothesis at alpha = 0.10 and power = 0.80, the required numbers of evaluable patients treated in each cohort are 49, 38, and 47, respectively, to demonstrate improvement in treatment outcome in these groups of at-risk pts.

The trial is currently enrolling and has an accrual goal of 148 patients.

**OT2-01-04**

**Cardiac Safety of Anthracycline-Containing Adjuvant Chemotherapy of Early Breast Cancer: OSCAR/ABC Ongoing, Observational, Multicentric Study.**

**Ricevuto E, Cocciolone V, Zilli M, Scognamiglio MT, Pistilli B, Di Menna G, Mancini M, Cattani K, Adinolfi MI, Ferrandina MG, Pancotti A, Recchia F, Latini L, Ficorella C, Iacobelli S. San Salvatore Hospital, University of L’Aquila, L’Aquila, Italy; SS. Annunziata Hospital and G. Bernabeo Hospital, University G. D’Annunzio, Chieti, Italy; Ospedale Civilie of Macerata, Macerata, Italy; E. Renzetti Hospital, Lanciano, Chieti, Italy; Catholic University of the Sacred Heart, Rome, Campobasso, Italy; Mazzini Hospital, Teramo, Italy; Ospedale Civilie of Avezzano, Avezzano, L’Aquila, Italy.

**BACKGROUND:** Among the different chemotherapeutic options available for adjuvant treatment of early breast cancer (EBC), anthracycline-containing regimens represent prevalent choices. OSCAR/ABC is an observational, prospective, multicentric study aimed at evaluating, in the clinical practice, the relevance of cardiac dysfunction and congestive heart failure (CHF) associated to “free choice”; anthracycline-containing adjuvant regimens and to identify patients at increased cardiac risk. **PATIENTS AND METHODS:** patients candidate to receive adjuvant anthracycline-containing chemotherapy, will be enrolled in the study. Data on demographic and clinical characteristics of patients (age, cardiovascular comorbidity) tumor features (TNM, histotype, ER and PgR status, Ki67, and HER2 status), type of adjuvant regimen and tolerability of treatment will be collected and centrally registered at the Consorzio Interuniversitario Nazionale per la Bio-Oncologia (CINBO) using e-CRFs. Data on type, sites and doses of radiotherapy performed will be collected, to evaluate the possible impact on cardiac function. Primary aim of the study is to evaluate the prevalence of cardiac dysfunction and CHF among the whole population enrolled, particularly according to risk criteria and type of administered chemotherapy. Cardiac dysfunction is defined as limiting cardio-vascular toxicity (≥ Grade 3 dyspnea, arrhythmia, hypertension) and/or asymptomatic LVEF reduction ≥20% (if >50% at the baseline) or ≥10% (if ≤50% at baseline); CHF is defined as clinical diagnosis and/or asymptomatic LVEF reduction ≥20% (if >50% at the baseline) or ≥10% (if ≤50% at baseline). Assessment of cardiac risk involves the evaluation of age (< vs ≥ 65 years), cardio-vascular comorbidities (requiring treatment or not) and Left-Ventricular Ejection Fraction LVEF (> vs ≤ 55%) at diagnosis. Clinical and instrumental cardiac evaluation (ECG, Echocardiography) will be performed at study entry, on-treatment and up to 5 years thereafter. Secondary objective of the study is to evaluate the clinical properness of different adjuvant anthracyclines-containing chemotherapy options, with regard to prevalence of therapeutic regimens, safety, efficacy (DFS and OS) and costs analysis. Cardiac safety and general toxicity on-treatment will be evaluated according to NCI criteria. The expected enrollment is 1,200 patients in 36 months. From September 2010 to May 2011, 7 of the 13 Centers involved in the study are active, with 65 enrolled patients.

**OT2-02-01**

**The SOLE Trial: International Breast Cancer Study Group (IBCSG 35-07) and Breast International Group (BIG 1-07) Study of Letrozole Extension.**

**Colleoni M. SOLE Collaborative Group and International Breast Cancer Study Group Group.**

**BACKGROUND:** The SOLE trial tests the hypothesis that treatment-free intervals during the course of five years of extended adjuvant letrozole will improve disease-free survival (DFS). The hypothesis is based on the observation in animal models that intermittent letrozole withdrawal will permit some estrogenic stimulation which makes residual resistant disease susceptible to letrozole reintroduction.

**Trial Design**

Randomized, Phase III trial comparing 5 years of continuous letrozole vs. 5 years of intermittent letrozole. The intermittent group receives letrozole daily for the first 9 months (followed by a 3-month gap) years 1-4, and 12 months year 5. Stratification factors are institution and prior endocrine therapy (SERM, AI or both).

**Major Eligibility Criteria**

-Postmenopausal
-Premenopausal
-Disease-free
-Received 4-6 years of prior adjuvant endocrine therapy (SERM and/or AI)
-Randomized within 12 months of last dose of prior endocrine therapy
-Endocrine-responsive breast cancer at diagnosis
-Node-positive breast cancer at diagnosis

**Specific Aim**

To compare continuous and intermittent letrozole given for a five year period for postmenopausal women who are disease-free following 4-6 years of prior adjuvant endocrine therapy with SERM(s) and/or AI(s) for endocrine-responsive node-positive operable breast cancer.

**Statistical Methods**

The primary analysis will be undertaken with the intention-to-treat population of all randomized patients. The primary endpoint DFS will be compared between treatment arms using a two-sided stratified logrank test with an overall experiment-wise alpha level equal to at most 0.05.

For the eligible population of patients with node positive disease at initial diagnosis, we assume 90% of patients who receive continuous extended letrozole will be alive and disease-free at 4 years from randomization (based on results of MA.17). The sample size was
determined to provide 80% power to detect a 20% reduction in the risk of an event defining DFS associated with intermittent letrozole compared with continuous letrozole (hazard ratio = 0.80; increase in 4-year DFS from 90% to 91.917%) using a two-sided 0.05 level test of significance.

**Present and Target Accrual**

Target: 4800; Present: 2655 (June 1, 2011)

**Related Research**

The SOLE-EST Substudy focuses on the changes in estrogen levels that occur on letrozole and during the three month treatment gap without letrozole; and relationships between changes in patient-reported musculoskeletal symptoms, grip strength and QL. The target accrual goal is 100 patients (75 intermittent and 25 continuous).

**The SOLE Quality of Life Substudy** compares differences in patient-reported symptoms and QL between continuous letrozole for 5 years and intermittent letrozole over a 2-year period. The target accrual goal is 834 patients.

**Central Pathology**

Review for all patients of histology, grade, ER, PgR, HER2, and Ki-67 is ongoing.

**Contact Information**

The SOLE trial is a worldwide collaboration among cooperative groups and large institutions primarily through the Breast International Group (BIG).

**OT2-03-01**

Incidence of Mastalgia as a Presenting Complaint in Iranian Population with Regard to Age, BMI, Education, Residency (City or Rural), State of Marriage and Compare with Western Countries.

Razavi S, Private Breast Clinic, Esfahan, Islamic Republic of Iran

As it is has usually been stated, breast pain (mastalgia) is among the most common (or the commonest) cause of women referring to a breast clinic. The incidence has been reported in different ranges, and some studies (e.g. Canadian groups) have stated that mastalgia has its lowest incidence in middle east (compared to western countries). Based on this information I have recently planned a study to investigate this issue.

To evaluate this data, I planned a questionnaire for every new patient coming to my breast clinic randomly. Every questionnaire was double checked & if still incomplete, was completed by phone communication.

The only inclusion criteria were to be a new patient. In this way over 550 questioners were completed during over 4 months. The aim is to find if there is any statistical difference in incidence rate and if there is any, evaluate any demographic difference that can be a cause.

**OT2-03-02**

Prospective Neo-Adjuvant Registry Trial Linking MammaPrint, Subtyping and Treatment Response: Neo-adjuvant Breast Registry – Symphony Trial (NBRST).

Whitworth P, Beitsh P, Gittleman M, Akbari S. Nashville Breast Center, Nashville, TN; Dallas Surgical Group, Dallas, TX; Breast Care Specialists, Allentown, PA; Virginia Hospital Center, Arlington, VA

**Background**

MammaPrint is performed on a diagnostic multi-gene array featuring >4,500 genes. This platform enables additional gene expression profiles to be analyzed simultaneously on one tumor specimen. BluePrint, an eighty gene Molecular Subtyping Profile, discriminates between three distinctive subtypes; Basal-type, Luminal-type, and ERBB2 (HER2)-type. Studies have shown marked differences in response to neo-adjuvant treatment in groups stratified by MammaPrint and BluePrint.

**Trial design**

A prospective observational, case-only study linking MammaPrint, BluePrint, TargetPrint, TheraPrint (together referred to as the Symphony suite) and possible additional profiles of interest to neo-adjuvant treatment response and Distant Metastases Free Survival (DMFS) and Relapse Free Survival (RFS). 20-30 institutions in the US will be invited to contribute clinical patient data from enrolled patients after a MammaPrint, TargetPrint, BluePrint and TheraPrint (Symphony suite) has been successfully performed and the patient has started neo-adjuvant therapy. Treatment is at the discretion of the physician, adhering to NCCN approved regimens or a recognized alternative.

**Eligibility criteria**

- Women with histologically proven breast cancer, who have started or are scheduled to start neo-adjuvant chemotherapy therapy or neo-adjuvant hormone therapy, after successful Symphony suite assessment
- Age 18-90
- Written informed consent
- No excisional biopsy or axillary dissection
- No confirmed distant metastatic disease
- No prior therapy for the treatment of breast cancer

**Scope**

The scope of this registry study is to measure chemosensitivity as defined by pCR (primary endpoint), or endocrine sensitivity as defined by partial response, (a primary endpoint for neo-adjuvant endocrine therapy and a secondary endpoint for neoadjuvant chemotherapy), metastasis-free survival and relapse-free survival (secondary endpoints) in molecular subgroups, determined by the MammaPrint and BluePrint; as well as correlation to Targetprint and Theraprint read outs in addition to investigating novel response profiles.

**Statistical methods**

The response rate and corresponding confidence intervals will be presented as a proportion of all patients enrolled. The confidence intervals will be calculated using the normal approximation to the binomial distribution. Comparison of response rates between different molecular subgroups will be conducted using Pearson Chi-square test. Correlation of chemosensitivity and endocrine sensitivity (as defined by pCR) to TheraPrint will be determined using Pearson correlation and linear fit models. Kaplan-Meier curves for RFS and DMFS will be calculated for different molecular subgroups.

**Present accrual and target accrual**

The target accrual is to enroll approximately 500 patients in 4 years.

**OT2-03-03**

Spectroscopic Feature of Breast Cancer.

Ogura H, Yamashita D, Nasu H, Hosokawa Y, Koizumi K, Yamaki E, Yoshimoto K, Suzuki T, Ueda Y, Oda M, Yamashita Y, Sakahara H. Hamamatsu University School of Medicine, Hamamatsu, Shizuoka, Japan; Hamamatsu Photonics K.K., Hamamatsu, Shizuoka, Japan

**Objectives:** To examine optical properties of breast cancer by time-resolved spectroscopy.

Materials and Methods: We irradiated a pulsed laser of 760, 800, and 830 nm wave-length lights at multiple sites of both breasts including the site just above the cancer and detected the light transmitted through the breast with TRS-20SH (Hamamatsu Photonics K.K.). Absorption coefficient (μa), reduced scattering coefficient (μs’), total hemoglobin...
(\text{tHb})$, and oxygen saturation (SO2) of the breast were calculated by photon diffusion equation. The clinical trial started in January 2007. A total of one hundred nine breast cancer patients participated in the trial and written informed consent were obtained from all of the patients. Results: In 800 nm wave-length, absorption coefficient (\(\mu_a\)) of breast cancer tissue was significantly high, compared with contra-lateral normal breast (cancer:0.0677±0.0293, normal breast:0.0479±0.0161, \(p<0.001\)).

**Table 1**

<table>
<thead>
<tr>
<th>(\mu_a) (cm(^{-1}))</th>
<th>(\mu_s) (cm(^{-1}))</th>
<th>(\mu_s') (cm(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>cancer</td>
<td>normal breast</td>
<td>cancer</td>
</tr>
<tr>
<td>860nm</td>
<td>0.076</td>
<td>0.0454</td>
</tr>
<tr>
<td>800nm</td>
<td>0.0677</td>
<td>0.0479</td>
</tr>
<tr>
<td>780nm</td>
<td>0.0823</td>
<td>0.0594</td>
</tr>
</tbody>
</table>

Absorption coefficient (\(\mu_a\)) and reduced scattering coefficient (\(\mu_s'\)) of cancer / normal breast

The result was the same in 760, and 830 nm. There was no difference in reduced scattering coefficient (\(\mu_s'\)) between breast cancer tissue and contra-lateral normal breast (800 nm cancer:9.07±1.217, normal breast:9.34±1.243, \(p=0.10\)). The \(\text{tHb}\) of breast cancer tissue was significantly high, compared with normal breast (cancer:31.0±14.7, normal breast:21.0±8.2, \(p=0.001\)). There was no difference in oxygen saturation (SO2) between breast cancer tissue and contra-lateral normal breast (cancer:72.8±4.1, normal breast:73.8±4.5, \(p=0.08\)).

Conclusion: Absorption coefficient (\(\mu_a\)) and \(\text{tHb}\) increased in breast cancer, whereas reduced scattering coefficient (\(\mu_s'\)) did not.

**OT2-03-04**

A Trial Model for the Future in the Search for Personalised Medicine – The UK POETIC and EPHOS-B Perioperative Trials Experience.

Bliss JM, Robison LE, Webster-Smith MF, Emson MA, Kilburn LS, Smith JE, Robertson J, Dowsett M, Bundred NJ, Cameron DA, Vidy R, Horgan K, Evans AA, Kokan JS, Pinhel I, A’Hern R, On Behalf of the POETIC & EPHOS-B Trialists. Institute of Cancer Research, Sutton, London, United Kingdom; Royal Marsden Hospital, London, United Kingdom; Nottingham University Hospitals, Nottingham, United Kingdom; Royal Marsden Hospital and Breakthrough Breast Cancer Centre, London, United Kingdom; Wythenshawe Hospital, Manchester, United Kingdom; Western General Hospital, Edinburgh, United Kingdom; Stafford Hospital, Stafford, United Kingdom; Leeds General Infirmary, Leeds, United Kingdom; Poole Hospital, Poole, United Kingdom; Macclesfield District General Hospital, Macclesfield, United Kingdom

**Background:** Perioperative therapy offers the opportunity to measure biological response to treatment in the primary tumor in early breast cancer (EBC), enhancing prospects for personalised medicine. Perioperative trials form an expanding component of the UK national breast cancer trials portfolio. Unlike traditional neoadjuvant studies, activity dovetails around timelines for standard treatment with no planned delay to primary surgery. Tissue samples collected prior to randomisation (baseline) & again at surgery address key biological endpoints & are essential for perioperative studies.

**Methods:** As the UK’s largest perioperative trial, POETIC (ER+ve postmenopausal EBC, +/- aromatase inhibitor therapy, biomarker & disease outcome) aims to recruit 4000 patients from 100+ centres. EPHOS-B (HER2+ve EBC, +/- lapatinib or trastuzumab) focuses on biomarker endpoints & aims to recruit 250 patients. Barriers to recruitment included 1) integration of research protocols into busy breast surgical clinics, extending a clinical trials culture across a multidisciplinary breast diagnostic team, & ensuring appropriate GCP training, 2) satisfying requirements for storing research tissue, 3) complying with government cancer wait times, 4) obtaining biomarker results within required timelines 5) ensuring completeness & quality of tissue samples. Additional challenges for EPHOS-B include managing requirements for scheduling of oncological therapy (e.g. trastuzumab) in pre-operative setting, delivery of such therapy outside the randomising hospital & rapid access to cardiac screening before randomisation.

**Results:** The following strategies were developed to overcome barriers 1) increasing collaborative working at sites & adopting a pragmatic approach to type of tissue required. Centres choose to provide both FFPE tissue & tissue in RNA-later, or FFPE tissue only; 2) working with national regulators to agree interpretation of current legislation in designation of when tissue is “in transit” (enabling it to reside outside a tissue bank) & “research” tissue (where transfer to a tissue bank is required); 3) agreement with government that procedures integral to perioperative trials comply with cancer wait times; 4) promoting reorganisation of site processes for obtaining essential biomarker results; 5) pilot lab work to inform site guidance on tissue collection procedures to ensure quality of samples received. A tracking database allows completeness of tissue samples to be monitored. Work to improve timelines for HER2 testing in EPHOS-B is ongoing, & challenges of delivering anti-HER2 therapy in this setting have been addressed.

**Conclusions:** Assessment of biological response to therapy in the primary tumor in EBC within national trials is feasible. Many barriers faced by POETIC have been overcome, & with recruitment now 100+ patients per month, newer centres benefit from earlier experience. Lessons learnt in POETIC apply to EPHOS-B, allowing investigators to focus on resolving more challenging issues specific to that trial. In many centres both trials have been important drivers in improving timeliness of molecular testing & therefore benefited the patient pathway in general as well as securing high quality trial data.

**OT2-03-05**

Evaluation of the Prevalence and Prognostic Significance of VEGF\(_{165b}\) in Breast Cancer Patients Compared to Healthy Women.

Guenthner-Biller MM, Rademacher A, Mayr D, Engelstädter V, Fries K, Jeschke U, Rack B. Ludwig-Maximilians-University, Munich, Bavaria, Germany

Background: VEGF\(_{165b}\) mRNA was first isolated in 2002 by RT-PCR out of renal cortex tissue which resulted in a shorter PCR product than predicted from previously identified isoforms. This isoform was subsequently identified and cloned in both primary epithelial cells as well as in stable immortalized podocyte cell lines. Because of the nature of this splice variant and its distal splicing of the 3’-untranslated region of the VEGF mRNA, most previously investigated expression studies will not have distinguished VEGF\(_{165b}\) but no in vivo data regarding the level of expression of VEGF\(_{165b}\) in breast cancer or its possible correlation with disease progression.

**Methods:** As the UK’s largest perioperative trial, POETIC (ER+ve postmenopausal EBC, +/- aromatase inhibitor therapy, biomarker & disease outcome) aims to recruit 4000 patients from 100+ centres. EPHOS-B (HER2+ve EBC, +/- lapatinib or trastuzumab) focuses on biomarker endpoints & aims to recruit 250 patients. Barriers to recruitment included 1) integration of research protocols into busy breast surgical clinics, extending a clinical trials culture across a multidisciplinary breast diagnostic team, & ensuring appropriate GCP training, 2) satisfying requirements for storing research tissue, 3) complying with government cancer wait times, 4) obtaining biomarker results within required timelines 5) ensuring completeness & quality of tissue samples. Additional challenges for EPHOS-B include managing requirements for scheduling of oncological therapy (e.g. trastuzumab) in pre-operative setting, delivery of such therapy outside the randomising hospital & rapid access to cardiac screening before randomisation.

**Results:** The following strategies were developed to overcome barriers 1) increasing collaborative working at sites & adopting a pragmatic approach to type of tissue required. Centres choose to provide both FFPE tissue & tissue in RNA-later, or FFPE tissue only; 2) working with national regulators to agree interpretation of current legislation in designation of when tissue is “in transit” (enabling it to reside outside a tissue bank) & “research” tissue (where transfer to a tissue bank is required); 3) agreement with government that procedures integral to perioperative trials comply with cancer wait times; 4) promoting reorganisation of site processes for obtaining essential biomarker results; 5) pilot lab work to inform site guidance on tissue collection procedures to ensure quality of samples received. A tracking database allows completeness of tissue samples to be monitored. Work to improve timelines for HER2 testing in EPHOS-B is ongoing, & challenges of delivering anti-HER2 therapy in this setting have been addressed.

**Conclusions:** Assessment of biological response to therapy in the primary tumor in EBC within national trials is feasible. Many barriers faced by POETIC have been overcome, & with recruitment now 100+ patients per month, newer centres benefit from earlier experience. Lessons learnt in POETIC apply to EPHOS-B, allowing investigators to focus on resolving more challenging issues specific to that trial. In many centres both trials have been important drivers in improving timeliness of molecular testing & therefore benefited the patient pathway in general as well as securing high quality trial data.
existing clinical data. There are two groups of patients included in this study; patients with newly diagnosed breast cancer and healthy volunteers.

Eligibility criteria: The eligibility criteria for the breast cancer group are newly diagnosed ductal, lobular or inflammatory breast cancer at stage I-IV, no prior treatment, above 18 years of age. The eligibility criteria for the healthy volunteers are healthy women with no history of cancer with no current medical therapies and above 18 years of age.

Specific aims: The primary objective of this trial is to estimate the prevalence of VEGF_165b in breast cancer patients and healthy volunteers. The secondary objective is the correlation of VEGF_165b with clinical characteristics over time.

Statistical method: The statistical analysis being used in this study will be primarily descriptive with the calculation of mean and median and confidence intervals. The difference in distribution of the values will be examined in a one way variant analysis. We will also correlate results from different biospecimens and correlate the prevalence of variant presence or absence.

Material and methods: The samples will be analysed using a specific ELISA as well as immunohistochemistry. The accuracy and sensitivity of the available ELISA are of utmost importance in this setting. Bates et al have done previous work evaluating the ELISA used in this study and have shown that it underestimates the value for VEGF_165b but that it is specific for VEGF_165b. Since we will rather look at individual variation over the course of time, it will not have an effect regarding the expected results but it prohibits a direct comparison of the measured levels of VEGF_165b to VEGF_165b if they are not corrected for this lack in sensitivity.

OT2-03-06

Hylton NM, Partridge SC, Rosen M, Kim E, L’Heureux DZ, Edsman L, University of California at San Francisco, San Francisco, CA; University of Washington, Seattle, WA; Hospital of the University of Pennsylvania, Philadelphia, PA; Brown University, Providence, RI; American College of Radiology Imaging Network, Philadelphia, PA

Background: Functional MRI techniques may be utilized for characterizing breast tumors and measuring response to neoadjuvant chemotherapy (NAC). Dynamic contrast enhanced (DCE) MRI is the most common functional breast MRI technique. Fitting DCE MRI data to an appropriate pharmacokinetic model allows noninvasive, in vivo measurement of physiological parameters related to tissue perfusion, microvascular permeability, and extracellular/extravascular volume fraction. Diffusion weighted imaging (DWI) MRI is an alternative technique that measures the mobility of water molecules in vivo and is sensitive to tissue characteristics such as cell density, membrane permeability, and microstructure. DWI provides complementary information to DCE MRI about tumor biology and has shown promise for early prediction of response. The master ISPY2 multi-center study is a process targeting the rapid, focused clinical development of paired oncologic therapies and biomarkers. Its framework is an adaptive phase II clinical trial design in the neoadjuvant setting for women with locally advanced breast cancer. As a sub-study, ACRIN 6698 will combine both DCE and DWI MRI data to generate novel imaging biomarkers that correlate with treatment response. The two studies will provide a rich data set that can be used to elucidate molecular pathways and tumor responses to novel investigational drugs with standard chemotherapy. **Trial design**: ACRIN 6698 will perform advanced DCE and DWI MR imaging as part of the I-SPY TRIAL. The ISPY 2 adaptive therapy design will use different tumor biomarker assays to identify patients with high risk of recurrence. Patients will receive NAC doublet chemotherapy and trastuzumab (if Her2+). Patients will be randomized and stratified into different arms receiving investigational agents of different drug classes. ACRIN 6698 patients will receive four advanced MRI exams (both DCE and DWI) at baseline, early therapy, mid therapy and prior to surgery.

**Specific aims**: The primary aim will determine if the % change in tumor apparent diffusion coefficient (ADC) measured on DWI from baseline to early treatment timepoint is predictive of pathologic complete response (pCR). The secondary aim will determine if the combined measurement of percentage change in tumor ADC on DWI, and percentage change in tumor volume and peak signal enhancement ratio (SER) on DCE MRI is predictive of pCR. **Statistical methods**: Receiver operating characteristic (ROC) curve and corresponding area under the ROC curves (AUC) for the individual marker, % change in tumor ADC, and % change in tumor volume and peak SER, will be estimated. Linear score of the 3 markers will be derived by fitting the multivariate logistic regression model, where the outcome is a binary variable for pCR and the predictors are the 3 measurements. The ROC curve for the derived linear score will be constructed and its AUC value will be estimated. **Target accrual**: ACRIN 6698 is open to ISPY 2 sites. The target accrual is 200 of ISPY 2’s planned enrollment of 800 participants.

OT2-03-07
Withdrawn by Author

OT2-04-01
A Randomized Phase II Trial of Vitamin D vs Placebo in Premenopausal Women: CALGB 70806.

Wood ME, Kingsley F, Ambaye AB, Yee L, Jung S-H, Marshall JR, Paskett E. University of Vermont, Burlington, VT; Ohio State University; Roswell Park Cancer Institute; Duke University

**Background**: Chemoprevention studies are long and expensive. Gaining information about the feasibility of an agent using biomarker endpoints can save money and ensure that only the most promising agents move forward. There is strong evidence to suggest breast cancer prevention properties for vitamin D. The safety and tolerability of this agent makes it a perfect candidate for a chemopreventive agent. This clinical trial will evaluate the effect of vitamin d on several breast cancer; providing important information regarding the feasibility of a larger chemoprevention study using cancer development as the endpoint. Successful completion of this study will also validate this design of biomarker testing of potential chemoprevention agents within a cooperative group setting and add to data validating these specific biomarkers.

**Methods**: This is a randomized placebo controlled trial of one year of 2000 units of vitamin D (cholecalciferol) for premenopausal women (≥ 35 years) with increased breast density. The primary aim of this study is to evaluate the effect of vitamin D on breast density. Secondary aims include evaluation of vitamin D effects on IGF1 and markers of cellular proliferation (Ki-67 and atypia). In addition 3 methods of density analysis (Breast Imaging Reporting and Data System (BI-RADS), Visual Analogue Scale (VAS), and the Boyd method) will be compared. For the primary endpoint of change in mammographic density we will have an 87% power to detect a standardized effect size (difference in means divided by standard deviation) of 0.4 in change of mammographic density between...
two arms. The target effect size is 2.64%. This study was activated 10/15/2010 and has been opened at 75 sites with 15 patients enrolled. Target accrual is 250.
Funding: This study is sponsored by the NCI and CALGB.

**OT2-04-02**  
**Metformin for Breast Cancer Prevention: A Pilot Study.**  
Ledgerwood MM, Ojeda-Fournier H, Patterson R, Hastei F, Andre MP, Cadmus L, Blair S. University of California, San Diego, CA

**Background:** There is a body of accumulating evidence connecting metformin, a biguanide derivative which reduces hepatic gluconeogenesis and improves skeletal muscle insulin sensitivity, to reduced breast cancer risk. This, in addition to a well-established drug safety record among diabetics and non-diabetics, has provoked an interest in further study of metformin’s effects on breast cancer risk indicators. In our study, we examine the effects of metformin on breast density and proliferative cell marker Ki67, both indicators of breast cancer risk. This is a small observational pilot study to determine the feasibility of subject recruitment, data collection through fine-needle aspiration, and breast density measurement.

**Trial Design:** Recruitment of 10 breast surgery patients with high risk histology to: 1. Undergo peri-areolar fine needle aspiration (RPFNA) used to collect breast cells for analysis of proliferative marker Ki67. 2. Breast density assessment by a) mammography (the standard method) and b) whole-breast ultrasound (an innovative method recently developed at UCSD).

Each subject will undergo a single view mammogram, whole-breast ultrasound, and intra-operative RPFNA. No drug or therapeutic intervention will take place in this pilot study.

**Eligibility:** Female undergoing excisional high risk breast surgery.

**Specific Aims:** Aim 1. To determine the feasibility of recruiting high risk breast surgery patients to an observational study involving RPFNA, mammography, and whole-breast ultrasound. Aim 2. a) To compare mammography and whole-breast ultrasound for measurement of breast density. b) To examine potential for detecting biomarkers of cell proliferation through RPFNA.

**Statistical Method:** Breast density will be determined by the ratio of fibroglandular tissue to fat measured on mammogram and WBU. Cell proliferation will be assessed by the percentage of positivity for biomarkers of cell proliferation through RPFNA.

**Accrual and target accrual:** Five of ten breast excisional high risk breast surgery subjects have been accrued at this time.

**OT2-04-03**  
**Uptake of a Randomized Breast Cancer Prevention Trial Comparing Letrozole to Placebo in BRCA1/2 Mutation Carriers:**  
The FNCLCC ONCO-03/LIBER Trial.

Pujol P, Lasset C, Berthet P, Dugas C, Delalorge S, Fricker JP, Chabbert Buffet N, Lemonnier J, Roca L, Mijonet S, Baudry K, Martin AL. University Hospital CHU Arnaud de Villeneuve, Montpellier, France; Centre Léon Bérard, Lyon and University of Lyon 1; CNRS; UMR 5558, Villeurbanne, France; Centre François Baclesse, Caen, France; Institut Gustave Roussy, Villejuif; Centre Paul Strauss, Strasbourg; AP HP Hôpital Tenon; Fédération Nationale Française des Centres de Lutte contre le Cancer

**Background:** Women with germline BRCA1 or BRCA2 (BRCA1/2) mutations are an extreme risk population for developing breast cancer, with a life-time risk of 56-80%. Prophylactic mastectomy provides a valid option to reduce such risk, but it considerably affects the quality of life. Medical prevention by aromatase inhibitor that has been recently shown to have preventive effect may thus be an alternative.

LIBER is an ongoing, double-blind, randomized phase III trial to evaluate the efficacy of five-years letrozole versus placebo to decrease breast cancer incidence in post-menopausal BRCA1/2 mutation carriers (NCT00673335). We present data on the uptake of this trial. Methods: To evaluate theoretical feasibility, we compared inclusion criteria of women in the LIBER trial (n=113) to characteristics of women entered in the prospective ongoing national GENEPSO cohort (n=1505). Uptake was evaluated through a survey sent to all active centres, with responses obtained from 17 of the 20 (85%) centres. Results: According to characteristics of the women included in GENEPSO cohort (n=1505) and the survey, approximately one third of BRCA1/2 mutation carriers are eligible for this study. From November 2009 to May 2010, 534 women eligible from chart review have been informed by mail of the trial and were invited to an oral information by participating centres. Of them, 44% of women came to the dedicated medical visit. Uptake of drug prevention trial was 32% of orally informed women and 15% of overall eligible women. Main reasons of refusal were: potential side effects, probability to receive the placebo and lack of support from the women’s physicians. Prior prophylactic oophorectomy and history of previous unilateral breast cancer were more frequent in women enrolled in the ONCO-03/LIBER trial than in the French cohort (93% versus 60% and 50 % versus 39 %, respectively), suggesting a higher motivation for medical prevention in these subgroups of patients. Conclusion: One third of women with a BRCA1/2 mutation are eligible to the ONCO-03/LIBER prevention trial. 32 % of orally informed women and, only 15% of overall eligible women entered the trial. To reach accrual objective (n=308), a greater information of the trial should be offered to women with BRCA1/2 mutation and the trial has been proposed to other countries.
brain metastasis or without brain metastases. Two positron emission tomography (PET) scans will be performed in each patient following intravenous administration of a microdose of [11C] lapatinib: in lapatinib-naïve patients and at steady-state lapatinib, after treatment with unlabelled oral lapatinib 1500 mg once daily for 8 days. [11C] lapatinib time-activity curves will be generated for normal brain and brain metastases, the PET volume of distribution in the brain calculated and penetration of [11C] lapatinib into the brain quantified. Analysis of brain PET data is explorative as [11C] lapatinib is a new tracer. Therefore, this study investigates both the difference in lapatinib brain penetration between patients with and without brain metastases, as well as the effect of low and high concentrations of lapatinib on the BBB efflux system. The study is currently recruiting in one center in the United Kingdom.

OT2-05-02
ACRIN 6691 Monitoring and Predicting Breast Cancer Neoadjuvant Chemotherapy Response Using Diffuse Optical Spectroscopic Imaging (DOSI).

Tromberg BJ, L’Heureux DZ, Mankoff DA, Zhang Z, Cervusi A, Mehta R, Carpenter PM, Butler JA, Hylton NM, Kaufman P, Pogue BW, Paulsen K, Yodh AG, Boas D, Isakoff S. University of California, Irvine, CA; American College of Radiology Imaging Network, Philadelphia, PA; University of Washington, Seattle, WA; Brown University, Providence, RI; University of California at San Francisco, San Francisco, CA; Dartmouth University, Lebanon, NH; University of Pennsylvania, Philadelphia, PA; Massachusetts General Hospital, Charlestown, MA; Massachusetts General Hospital, Boston, MA

Background: Imaging technologies monitoring and predicting breast cancer response to neoadjuvant chemotherapy (NAC) are of increasing interest. The utility of conventional imaging approaches varies and identifies the need for alternate functional imaging strategies. The use of model-based photon migration methods to quantitatively separate light absorption from scattering in multiply-scattering tissues is a type of near-infrared spectroscopy (NIRS) broadly referred to as diffuse optical spectroscopy (DOSI) [Buvlewac, et al. Applied Optics, 2000; Jakubowski, et al., J of Applied Optics, 2009]. DOSI is a promising experimental technology that allows patients undergoing NAC to be followed with a “no significant risk” device meeting Food and Drug Administration criteria for exempt status. The current design is a mobile device which offers increased accessibility and is relatively simple to perform and interpret, as compared to mammography, magnetic resonance imaging, and positron emission tomography. Due to its size and portability, DOSI is a low barrier-to-access technology, creating new opportunities for patients to receive personalized treatment and for physicians to gain new insight into response mechanisms. The long-term goal is to provide oncologists with a relatively simple, risk-free bedside tool that can be used to help inform medical decisions on chemotherapy regimen, duration, and timing of surgery, thereby maximizing therapeutic response and minimizing unnecessary toxicity. Trial design: In this phase I/II prospective single arm study, patients will receive SOC NAC at five (5) NCI Network for Translational Research in Optical Imaging (NTROI) clinical sites with identical DOSI instruments and procedures. Patients will receive four DOSI exams: at baseline before chemotherapy, at early therapy 5-10 days after NAC initiation, at mid therapy, and at post therapy prior to surgery. The protocol will evaluate a harmonized DOSI technology platform that has been standardized for NAC monitoring. Eligibility: Women who have been diagnosed with breast cancer, have had confirmation by pre-treatment biopsy, and are scheduled to receive NAC followed by surgery are eligible for this trial. Specific aims: The primary aim of this clinical trial is to determine whether the baseline to mid-therapy changes in the DOSI measurement of the quantitative tumor tissue optical index can predict final pathologic complete response in patients with breast cancer undergoing NAC. The secondary aims investigate the correlation between additional DOSI quantitative measurements of tumor biochemical composition obtained at other timepoints, the full range of pathologic response (i.e. complete, partial, and non-response), and any corresponding imaging measurements. Statistical methods: Logistic regression models will be used to study the relationships between pathological complete response and percent change in tissue optical index tumor to normal ratio at different imaging time points. Study size: A total of sixty (60) patients will be enrolled in this imaging study. Currently, one patient has accrued.

OT2-05-03
ACRIN 6688 Phase II Study of Fluorine-18 3’-Deoxy-3’ Fluorothymidine (FLT) in Invasive Breast Cancer.

Jolles PJ, Kostakoglu L, Bear HD, Idowu MO, Kurdziel K, Shankar L, Mankoff DA, Duan F, L’Heureux DZ. Virginia Commonwealth University, Richmond, VA; Mount Sinai School of Medicine, New York, NY; National Cancer Institute, Bethesda, MD; University of Washington, Seattle, WA; Brown University, Providence, RI; American College of Radiology Imaging Network, Philadelphia, PA

Background: Neoadjuvant chemotherapy (NAC) prior to surgery provides enhanced options for locoregional management and has become an integral component of primary breast cancer management. Initial tumor response in patients receiving NAC is generally determined at therapy completion. This evaluation is determined by either the presence/absence of palpable tumor as a clinical response and/or presence/absence of invasive tumor cells in the breast and nodes as a pathological response. The ability to evaluate the effectiveness of neoadjuvant therapy early during treatment would be of significant importance. FDG PET imaging has been shown to be predictive of subsequent tumor response, but the tendency of FDG to accumulate in inflammatory tissues can complicates image interpretation. MRI changes have also been touted as predictors of response. Preliminary data suggest that early FLT PET is better able to predict response to therapy, as FLT uptake has been shown to correlate with cellular proliferation, and does not significantly accumulate in inflammatory tissue (Kenny et al, Eur J Nucl Med Mol Imaging 2007:1339-1347). The analysis of these data may provide a better understanding of early treatment response and improve the clinical management of breast cancer in the future. Trial design and eligibility: In this phase II multi-institutional study, breast cancer patients with locally advanced disease with a tumor size ≥2cm (measured on imaging or estimated by physical exam) are eligible. Participants will receive standard of care NAC at their respective institutions. Participants will have 3 FLT imaging sessions to evaluate therapy response: at baseline, early-treatment (5-10 days after initiating treatment), and post-treatment prior to surgery. Specific aims: The primary objective is to correlate the percentage change in the standardized uptake value between baseline and early therapy FLT in the primary tumor with pathologic response. Correlatively, FLT PET parameters will be compared with proliferative indices from the initial biopsy and residual tumor surgical samples using Ki-67 staining, mitotic index, and residual cancer burden. Potential safety issues and the physiologic effects associated with FLT administration will also be evaluated. Statistical methods: To evaluate the relationship between an uptake parameter and pathologic complete response, a ROC curve
will be estimated and the area under the curve, along with its 95% confidence interval, will be determined. **Accrual:** Currently, 45/67 patients have accrued to the study.

### OT2-05-04

**ACRIN PA 4006: Comparison of Full-Field Digital Mammography with Digital Breast Tomosynthesis Image Acquisition in Relation to Screening Call-Back Rate.**

Conant EF, Maidment A, Copit D, Olson CB, Heckel ML, Gatsonis C. Hospital of the University of Pennsylvania, Philadelphia, PA; Albert Einstein Medical Center, Philadelphia, PA; American College of Radiology Imaging Network, Philadelphia, PA; Brown University, Providence, RI

**Background:** With competing parameters of specificity and sensitivity, breast cancer screening must limit both missed cancers and false-positive call-backs (with potential to biopsy) to reduce cost and unnecessary anxiety to patients. Full-field digital mammography (FFDM) has been shown to provide improved sensitivity over analog mammography in the detection of breast cancers, particularly in women with dense breasts. Unfortunately, call-back rates for both digital and analog screening mammography have similar, elevated rates of approximately 10% (E. Pisano et al, NEJM, 2005). Incorporation of digital breast tomosynthesis (DBT), a novel 3-D reconstruction of multiple low-dose digital mammographic images, may reduce the number of call-back visits needed by providing many of the benefits of diagnostic imaging at screening. This study will assess variations in DBT image acquisition while limiting radiation dose to women with varying breast sizes and densities, to define parameters to be used in a larger national screening tomosynthesis trial.

**Eligibility:** Asymptomatic women 25 years and older with no breast cancer history are eligible for Group A or B (see below). Pregnant women, women unable to tolerate compression of the breast associated with mammography, women with implants, and women with breasts too large for DBT are excluded from participation. **Trial design/target accrual:** This multicenter trial uses Hologic digital mammography units at two institutions in Philadelphia, Pennsylvania. The timing of the study-related imaging visits are segregated into two cohorts, screening (Group A) and diagnostic (Group B). Group A comprises 500 women who will undergo both FFDM and DBT at screening. Initial independent interpretations of the FFDM and DBT from local readers will determine call back. Enriched Group B comprises 50 women who have been informed of abnormal findings from a FFDM screening within 30 days of enrollment. They will be recruited prior to their diagnostic imaging and consent to DBT of both breasts as part of their diagnostic imaging. Participants from both Groups will be followed or biopsied as recommended by their treating physician. Follow up includes medical record review and images collection at approximately 1-year. Follow-up data may be collected up to 18 months. **Specific aims and statistical methods:** ACRIN PA 4006 will evaluate the specificity of 2-D FFDM versus a combination of 2-D and 3-D tomosynthesis imaging in breast cancer screening. Specificity will be measured by the participant call-back rate by each modality. Sensitivity and specificity by lesion-type characteristics, especially focused on DBT assessment of calcifications, will be compared. **Current accrual:** Currently, the trial accrual has reached 259 of 550.

### OT2-05-05

**Phase I/II Study of Adoptive T Cell Therapy Following In Vivo Priming with a HER2 Peptide-Based Vaccine in Patients with Stage IV Breast Cancer.**

Parker SL, Higgins DM, Childs JS, Dang Y, Guthrie KA, Disis ML, Salazar LG, Coveler A. University of Washington, Seattle, WA; Fred Hutchinson Cancer Research Center, Seattle, WA

**Background:** Adoptive T cell therapy has evolved from preclinical setting to a potentially feasible treatment strategy for advanced breast cancer (BC). However, the ability to expand tumor antigen specific T cells ex vivo has been one of the major hurdles that has limited clinical translation of adoptive T cell therapy. Tumor specific T cells are rare in unprimed patients and generating large bulk cultures from rare precursor frequencies is difficult. We have found immunizing HER2+ patients to increase tumor specific T cell precursor frequencies to the levels of a vaccinated foreign antigen markedly improves the ability to generate large numbers of tumor specific T cells in vitro. We hypothesize that T cell expansion strategies that are facilitated by prior immunization will be clinically useful in the treatment of advanced BC.

**Design:** A Phase I/II non-randomized, single arm study. Priming with a HER2 ICD vaccine will be performed at 1 week intervals for a total of 3 vaccines. Patients will undergo leukapheresis 2 weeks after the 3rd vaccine to collect PBMC for T cell expansion. Patients will be pre-treated with cyclophosphamide 24 hours prior to 1st T cell infusion and then receive up to 3 dose-escalating infusions of T cells given 7-10 days apart. Three HER2 vaccine booster immunizations will then be administered at 1, 2, and 4 months after the final T cell infusion. Follow-up for persistent and continued immunity will then ensue.

**Aims:** To evaluate the safety of infusing escalating doses of HER2 specific T cells into patients with advanced HER2+ BC using ex vivo expanded autologous T cells, to investigate to what extent HER2 specific T cell immunity can be boosted or generated in individuals after infusion of HER2 specific T cells, to evaluate how long T cell immune augmentation persists in vivo after adoptive transfer of HER2 specific T cells and subsequent booster immunizations.

**Criteria:** Patients with HER2+ Stage IV BC who: have been maximally treated and not achieved a complete remission, have stable or slowly progressive disease, HER2+, and have adequate LVEF.

**Statistical Methods:** Toxicity will be determined by chemical and clinical parameters evaluated at various time points. If the true probability of a DLT is 0.11, then the probability of observing 0 DLT’s in 20 patients is 0.097. If the true probability of a DLT is 0.18, then the probability of observing ≤1 DLT’s in 20 patients is 0.102, and if the true probability of a DLT is 0.27, then the probability of observing ≤2 DLT’s in 20 patients is 0.064. Therefore, with low observed rates of DLT (<10%), we can be reasonably confident (~90%) with 20 patients that the true DLT rate is < 0.27. Immunologic response, defined as the successful boosting of precursor frequency with infusion of HER2-specific T cells, will be evaluated by assessing the change in T cell level from baseline. To assess the durability of the T cell response, we are primarily interested in estimating the proportion of patients whose T cells persist at a level the same or greater as the level after the final T cell infusion as long as 6 months following the final booster vaccine.

**Accrual:** Target=20/Actual=14
**OT2-05-06**

ACOSOG Z11101/ACRIN 6694: Effect of Preoperative Breast MRI on Surgical Outcomes, Costs and Quality of Life of Women with Breast Cancer.

**Bedrosian I, Suman VJ, Yao K, Shih Y-CT, Yen TWF, Comstock C, Newstead G, Birdwell R, Kim E, L’Heureux DZ, Gatsonis C. MD Anderson Cancer Center, Houston, TX; Mayo Clinic, Rochester, MN; University of Chicago, Evanston, IL; University of Chicago, Chicago, IL; Medical College of Wisconsin, Milwaukee, WI; Memorial Sloan-Kettering Cancer Center, New York City, NY; Harvard Medical School, Boston, MA; Brown University, Providence, RI; American College of Radiology Imaging Network, Philadelphia, PA**

**Background:** Surgical planning and local-regional treatment of breast cancer relies on accurate assessment of disease extent including the primary tumor size and the presence/absence of multiple tumor foci. As a staging modality for breast cancer, MRI has shown high sensitivity for detection of additional foci of diseases within the index breast. However, the impact of preoperative breast MRI on reducing re-excision rates and improving local control is less clear. Data from the COMICE trial in the UK suggested that routine use of pre-operative breast MRI did not alter rates of re-excision; however issues have been raised about the lack of quality standards for the MR imaging that may have resulted in the negative results of this trial. Retrospective data suggest that local recurrence is not impacted by use of breast MRI. In concert with data showing no improvement in clinical outcomes of breast cancer patients, concerns have been raised that routine use of preoperative breast MRI is associated with increased rates of mastectomy and delays to surgery. Therefore, the application of MRI for preoperative surgical staging remains controversial. In order to address this ongoing controversy, a joint effort has been launched by ACOSOG and ACRIN for a prospective clinical trial focused on evaluating the impact of preoperative breast MRI on clinically relevant patient outcomes. An important part of this collaboration is implementation of standards of how MRI findings should be clinically managed and used to direct localization methods and surgical planning, thereby creating guidelines for subsequent patient intervention. **Trial design/eligibility criteria:** A prospective multicenter trial will include women eligible for BCT by standard criteria and randomized between current standard of care, clinical examination and mammography (+/- ultrasound) and the same plus preoperative breast MRI. The study will focus on women at the highest risk of local recurrence: ER/PR/HER-2 negative (triple negative) and HER-2 amplified breast cancers. Specific aims: To compare the rates of local recurrence following breast conserving therapy in a cohort randomized to preoperative staging with mammography or mammography plus breast MRI. Additionally, a comparison of rates of re-operation, time to local recurrence, survival outcomes, contralateral breast cancer rates, rates of multicentric disease and other secondary aims will be performed. Costs and quality of life measures will also be investigated. **Statistical methods:** A stratified logrank test and Cox partial likelihood score test will be used to assess whether the distribution of LR times differs with respect to diagnostic work-up approach having adjusted for tumor stage. Cox modeling with the Cox partial likelihood score test will be used to examine the strength of association between these time to event distributions and such additional potential prognostic factors as menopausal status, chemotherapy, radiation therapy, ER, PR, number of positive lymph nodes, HER-2/neu expression, Nottingham grade, and Ki-67 expression. **Target Accrual:** 556 patients

**OT2-06-01**

A Phase III Randomized Multicentric French Study To Evaluate the Impact of a Localized 16-Gy Boost after Conservative Surgery and a 50-Gy Whole-Breast Irradiation in Breast Ductal Carcinoma In Situ (The BONBIS Trial).

**Azria D, Cowen D, De La Lande B, Bourjorgier C, Latorzeff I, Leblanc-Onfroy M, Douadi-Gaci Z, Pradier O, Peignaux K, Levy C, Ellis S, Lecouillerd I, Racadot S, Bontemps P, Benyoucef A, Lagarde P, Laharie-Mineur H, Cretin J, Marchal C, Serin D, Lemanski C. CRLC Val d’Aurelle, Montpellier, France; AP-HM, Marseille, France; Centre Rene Huguenin, Saint Cloud, France; IGR, Villejuif, France; Groupe ONCORAD, Toulouse, France; Centre Rene Gauducheau, Nantes, France; Centre Catherine de Sienne, Nantes, France; CHU Brest, Brest, France; CRLC G-F Leclerc, Dijon, France; CRLC Francois Baclesse, Caen, France; Centre Catalan d’Oncologie, Perpignan, France; CRLC Eugene Marquis, Rennes, France; Centre Leon Berard, Lyon, France; CHU Besancon, Besancon, France; Centre Henri Becquerel, Rouen, France; Institut Bergonie, Bordeaux, France; Clinique Tivoli, Bordeaux, France; Clinique Valdegour, Nimes, France; Centre Alexis Vautrin, Vandoeverlees Nancy, France; Institut Sainte Catherine, Avignon, France**

**Background:** Ductal carcinoma in situ is defined as breast cancer confined to the ducts of the breast without evidence of penetration of the basement membrane. Local treatment quality represents one of the most prognostic factors as half of recurrences are invasive diseases. The main goal of adjuvant radiotherapy after conservative surgery is to decrease local recurrences and to permit breast conservation with low treatment-induced sequelae. Several randomized trials have established the impact of 50 Gy to the whole breast (WB) in terms of local control. Nevertheless, no randomized trial is still available concerning the role of the boost in this disease. The phase III randomized trial “BONBIS” is elaborated to evaluate the impact of a 16-Gy boost after 50 Gy delivered to the whole breast in 25 fractions and 33 days.

**Methods:** A total of 1950 patients DCIS breast cancer patients are planned to be enrolled in this trial. Patients will receive the following treatment: (A) WB radiotherapy of 50 Gy in 25 fractions vs. (B) WB radiotherapy of 50 Gy in 25 fractions plus a localized 16-Gy boost in 8 fractions. The primary endpoint is local-relapse free survival (LRFS). This trial is designed to detect an expected rate in control arm of 7% and 4 % in experimental arm. With 90% power and a=0.05, 137 events are necessary to achieve the main goal. An interim analysis is planned after 50% of observed event. Stratifications are made based on recognized prognostic factors: age, hormonal treatment, differentiation, circumstance of diagnosis, surgical margin, centre. Secondary endpoints are relapse free survival, overall survival, acute and late toxicities, cosmetic results, and quality of life. Translational researches are also planned to identify intrinsic radiosensitivity of normal tissues (radiation-induced apoptosis assay, genome-wide association study) but also predictive models of tumor recurrences. Inclusions have started in November 2008 and are not so far than the planned estimation.

This trial is granted by the French National Cancer Institute (PHRC 2008) and supported by the French National Society of Radiation Oncology (SFRO).
**OT2-06-02**

A Randomized Phase III Study of Conventional Whole Breast Irradiation (WBI) vs Partial Breast Irradiation (PBI) for Women with Stage 0, 1, or 2 Breast Cancer: NSABP B-39/RTOG 0413. Julian TB, Costantino JP, Vicini FA, White JR, Cecchini RS, Winter KA, Arthur DW, Kurke R, Rabinoivitch R, Parda DS, Mamounas EP, Curran, Jr WJ, Wolmark N. National Surgical Adjuvant Breast & Bowel Project (NSABP), Pittsburgh, PA; Allegheny General Hospital, Pittsburgh, PA; NSABP Biostatistical Center, Pittsburgh, PA; University of Pittsburgh Graduate School of Public Health, Pittsburgh, PA; Beaumont Health System, Royal Oak, MI; Radiation Therapy Oncology Group (RTOG), Philadelphia, PA; Medical College of Wisconsin, Milwaukee, WI; Virginia Commonwealth University, Richmond, VA; Arizona Breast Cancer Specialists, Scottsdale, AZ; University of Colorado Denver, Aurora, CO; Aultman Health Foundation, Canton, OH; Emory University, Atlanta, GA

**Background:** Breast-conserving therapy (BCT) with WBI is an accepted option in the treatment of most patients with stage 1 or 2 breast cancer, but not all eligible patients utilize it. This may be because of age, length of time required for WBI treatment and availability of this method, and burden of care. There is a large body of mature phase I and II data, and some preliminary phase III data, that relate to the replacement of WBI after lumpectomy with PBI delivered in 4-5 days and restricted to the region of the tumor bed. Can an acceptable outcome be achieved with radiation therapy (RT) delivered only to the region of the tumor?

**Trial design:** For the administration of WBI, we are using standard techniques delivered over 5-7 weeks as outlined in the B-39/0413 protocol. For the administration of PBI, we are using high-dose-rate multi-catheter brachytherapy, high-dose-rate single-entry intracavitary brachytherapy (MammoSite®, MammoSite® ML, Contura®, MLB, and SAVI®), and 3D conformal external beam RT. PBI is given twice a day, at least 6 hours apart, on 5 treatment days over a period of 5-10 days. The technique selected depends on technical considerations, radiation oncology facility technique credentialing, and patient preference. Before random assignment, patients must have a planning CT. Those randomized to WBI receive chemotherapy, if applicable, before their RT. Those randomized to PBI receive RT before chemotherapy, if applicable. All treatment sites must pass a rigorous QA and QC process for PBI before and during the duration of the study. We are using a QOL program to assess cosmesis, fatigue, symptoms, and perceived convenience of care.

**Eligibility criteria:** Patients must have stage 0 or stage 1 or 2 IBC with no evidence of metastatic disease. Tumor size must be 3 cm or less. Women must have undergone a lumpectomy with the margins of the resected specimen histologically free of cancer, including DCIS. Patients must have no more than 3 positive axillary nodes. Accrual is open to all premenopausal women and to postmenopausal women who have IBC that is ER/PR negative or node positive. Patients are stratified according to disease stage, menopausal status, hormone receptor status, and intention to receive chemotherapy. Following stratification, patients are randomly assigned to receive WBI or PBI.

**Specific aims:** The primary endpoint is the comparison of those receiving PBI and WBI for the diagnosis of in-breast tumor recurrence (IBTR). The study will also compare women receiving PBI and WBI for overall survival, recurrence-free survival, and distant disease-free survival. QOL aims are related to cosmesis, fatigue, treatment-related symptoms, and perceived convenience of care.

**Statistical analysis and accrual:** PBI and WBI will be declared equivalent if the estimated relative risk of IBTR falls between 0.86 and 1.17. Analysis will occur after 175 IBTRs are diagnosed. At the end of May 2011, accrual was 3962 of 4300 patients. Supported by PHS grants NCI-U10-CA-69651, NCI-U10-CA-12027, and NCI P30-CA-14599 from the US NCI.

**OT2-07-01**


**Background:** Lymphocele is the principal post-operative morbidity following axillary node dissection. According to the literature, incidence can vary from 4 to 89%. Encouraging results in terms of reducing postoperative lymphoceles as well as drainage duration and volume using octreotide have been recorded recently. A new molecule, namely pasireotide, developed by Novartis Pharma AG, Basle Switzerland, is a somatostatin analog possessing high binding affinity to 4 of the 5 somatostatin receptors. Trial design: We are performing a prospective, randomized 1:1, double blind, multicenter trial against placebo with a Bayesian design. Eligibility criteria: any female patient scheduled for breast surgery with mastectomy and axillary node dissection indicated at the pre-surgical stage. Specific aims: The purpose of this trial is to assess the efficacy of a single pre-surgical injection of pasireotide LAR 60 mg im in reducing the postoperative incidence of symptomatic lymphoceles following mastectomy with axillary node dissection for breast cancer. Patients are followed up for 4 weeks.

**Statistical methods:** The statistical analysis will be carried out sequentially after observing the principal criterion (i.e. success is defined as a total volume of lymphocele following single or repeated aspiration ≤60 cc in the 28 days following surgery or a routine aspiration volume on the 28th day ≤120cc) of each patient included for each randomization group, with or without treatment. It involves estimating the probability of a response in each group using a Bayesian design based on a beta-binomial model. With the Bayesian approach, the response rate in each group (\( \hat{p} \)) is considered as a random variable, with a priori density focused on the anticipated response rate of 80% in the group receiving treatment and 60% in the non-treatment group, which will be sequentially updated as the observations are made according to a so-called posteriori law. Present accrual and target accrual: The sample size consists of a total of 90 patients with 45 patients in the active treatment group and 45 patients in the placebo one. To date more than 50% of the patients have been included.
OT2-07-02
SWOG S0927: A Randomized Double Blind Placebo-Controlled Trial of Omega-3-Fatty Acid for the Control of Aromatase Inhibitor (AI)-Induced Musculoskeletal Pain in Women with Early Stage Breast Cancer.
Hershman DL, Unger JM, Crew KD, Moiinpour CM, Minasian LM, Hansen L, Lew DL, Kaberle K, Wade JL, Meyskens FL. Columbia University, New York, NY; SWOG, Seattle, WA; National Cancer Institute, Bethesda, MD; Legacy Good Samaritan Hosp & MC, Portland, OR; SWOG, San Antonio, TX; Cancer Care Specialists of Illinois, Decatur, IL; University of California, Irvine, Orange County, CA

Background: Despite the well-proven efficacy of aromatase inhibitors (AIs) for the treatment of hormone-sensitive breast cancer, a significant number of women suffer from musculoskeletal side-effects which can result in early discontinuation of this important medication. Given the proposed anti-inflammatory effects of omega-3-fatty acid and the paucity of therapeutic options for AI-induced arthralgia, it is therefore reasonable to test the efficacy of omega-3-fatty acid in women with breast cancer who have developed moderate to severe joint symptoms after initiating AIs.

Specific aims: To assess if omega-3-fatty acid as compared to placebo causes a reduction in worst joint pain/stiffness in women with AI-associated arthralgias at 12 weeks as measured by the modified Brief Pain Inventory (BPI). Additional measures will include the WOMAC, M-SACRAH, FACT-ES and global rating of change, which will be assessed at baseline, 6, 12 and 24 weeks. We will evaluate fasting lipids, hormone levels, serum inflammatory markers (TNF, IL2, CRP), and markers of joint destruction (CTX-II) at baseline, 12 and 24 weeks.

Eligibility criteria: Pts. must have histologically-confirmed stages I-III breast cancer, with no evidence of metastatic disease and undergone definitive breast cancer surgery. Pts must be post-menopausal and currently be taking a third-generation AI – anastrazole (Arimidex®), letrozole (Femara®), or exemestane (Aromasin®) for at least the previous 90 days prior to registration with plans to continue for at least an additional 180 days after registration. The patient must have a worse joint pain/stiffness score of 5 or greater on the 10-point scale of the BPI which started or increased after initiation of AI. Pts must not have taken omega-3-fatty acid supplements within the past 3 months prior to registration. Pts will be randomized to receive 6 capsules daily (at 1,000 mg each; ~600mg combination of ethyl esters EPA/DHA) of omega-3-fattyacid or matching placebo daily for 24 weeks.

Statistical methods: We stipulate an alpha=.05 two-sided test, with an estimated 5% non-adherence and 20% dropout rate at the primary endpoint evaluation time of 12 weeks after randomization. For a two point difference in worst joint pain/stiffness and a 3.5 point SD at 12 weeks, 222 eligible patients would be required for 90% power under a two-arm normal design. To allow ineligibility rate of 10%, 246 total pts will be enrolled. The study should be activated September 2011.

Funding: Supported by National Cancer Institute grant CA037429

OT3-01-01
Adelson KB, Raptis G, Sparano J, Germain D. Mount Sinai School of Medicine, New York, NY; Albert Einstein Cancer Center, Bronx, NY

Background: Bortezomib is a proteasome inhibitor that enhances fulvestrant-mediated aggregation of the ER in the cytoplasm without blocking ER degradation in the nucleus in ER+ human breast cancer cell lines, thereby promoting cytoplasmic ER aggresomes which activate a sustained unfolded protein response leading to apoptotic cell death; the combination also induces tumor regression in a tamoxifen resistant T47D-cyclin D1 xenograft model more effectively than either agent alone (Clin Cancer Res 2011; 17: 2292-2300).

Hypothesis: We hypothesize that the combination of fulvestrant and bortezomib will be more effective than fulvestrant alone in ER+, AI-resistant MBC.

Trial design: This is an open-label randomized phase II design in which patients with MBC are randomized to receive fulvestrant alone (500 mg IM day -14, day +1, and day +14 during cycle 1, then 500 mg every 4 weeks on day +1 during cycle 2 and thereafter) or in combination with bortezomib (1.6 mg/m2 IV days +1, +8, +15 every 28 days). Stratification factors for randomization include performance status (ECOG 0 vs. 1-2), measurable disease (yes vs. no), and prior chemotherapy for MBC (yes vs. no). Patients who progress on fulvestrant alone may cross over to the combination.

Eligibility criteria: Postmenopausal women with ER+, Her2-negative, MBC who have progressive disease during AI therapy for metastatic disease, or relapse while receiving adjuvant AI therapy. Up to one prior chemotherapy regimen for metastatic disease is permitted.

Specific aims:
Primary Objective: To determine if the addition of bortezomib to fulvestrant significantly improves median progression-free survival (PFS), defined as the time from cycle 1, day 1 of therapy until disease progression or death from any cause.

Secondary Objectives: To determine: (1) adverse event rates in both arms, (2) the clinical benefit rate (CBR – objective response [by RECIST 1.1] and/or progression free at 24 weeks), (3) the objective response and CBR after crossover from fulvestrant to fulvestrant plus bortezomib.

Translational Objectives: To perform an exploratory analysis of the effects of the combination on intratumoral nuclear/cytoplasmic ER ratio, unfolded protein response (BiP), apoptosis (cleaved caspase 3, Bcl-2 phospho JNK.)

Statistical methods: he median PFS for patients receiving fulvestrant alone is expected to be approximately 5.4 months based upon patients with AI-resistant disease enrolled on the CONFIRM trial ( J Clin Oncol 2010; 28: 4594-4600). The trial is designed to detect a 70% improved in median PFS to 9.0 months (alpha=0.10, beta =0.10), which will require 59 eligible patients in each arm.

Present accrual and target accrual: 24/118
OT3-01-02
Imatinib Mesylate in Combination with Vinorelbin for Patients with Metastatic Breast Cancer – An Ongoing Phase I/II Clinical Trial.
Mundhenke C, Schem C, Bauerschlag DO, Weigel MT, Hilpert F, Eidmann H, Tiemann K, Muth M, May C, Hanson S, Jonat W, Maass N, Kiel University, Kiel, SH, Germany; RWTH Aachen, Aachen, NRW, Germany; Novartis Pharma, Nuremberg, Bavaria, Germany; Institut fuer Haematopathologie Hamburg, Hamburg, HH, Germany

Background: Imatinib mesylate is a tyrosine kinase inhibitor which originally had been developed to block pathognomonic bcr-abl oncprotein in chronic myeloid leukemia. It is also an inhibitor of the receptor tyrosine kinases of platelet-derived growth factor (PDGF) and stem cell factor (SCF), c-Kit, and abl. Therefore it also inhibits PDGF- and SCF-mediated cellular events, as tumor cell proliferation in solid tumors. Additionally, inhibition of PDGF- b-receptor relieves tumor hypertension in solid tumors and thereby helps to deliver higher concentrations of cytotoxic drugs into the tumor cell. Vinorelbin is a semisynthetic vincaalcaloid with antitumor activity. As a single agent and as a combination partner of other cytotoxic drugs, it has proven good tolerability and high effectiveness in treatment of patients with metastatic breast cancer (MBC). This study was designed to evaluate the feasibility of the combination of imatinib mesylate and the cytotoxic drug of vinorelbin in different concentrations in patients with advanced and MBC.

Study design: This is a prospective open-label, single arm phase I/II study combining 400 mg Imatinib Mesylate p.o. daily (amended from 600 mg) with an escalating dose of vinorelbin i.v. weekly for patients with locally advanced or metastatic breast cancer. Patients must have tumors expressing PDGF-receptor-α and/or -β and/or c-kit and have received pretreatment with an anthracyclin containing regimen. Main study endpoints are feasibility and tolerability of this novel combination. In addition to the total toxicity and the incidences of hematological and non-hematological toxicity of grade 3 and 4, the clinical activity together with the clinical response rate and the time to disease progression will be evaluated. The quality of life is examined during the whole course of treatment. Recruitment started for level one with 10 mg/m² vinorelbin and proceeds to the next higher levels 15, 20 and 25 mg/m². Each dose level will be filled with at least five patients. Patients of one dose level will be followed for a minimum of 28 days during therapy, before enrolment of patients for the next dose level can start. For translational research multiple skin biopsies will be taken (skin wounding assays). When feasible tumor-biopsies are taken before and during therapy. Expression of tyrosine kinase receptors (c-kit and PDGF-receptor) will be correlated with treatment response.

Present accrual and target accrual: 33 patients have been enrolled into the study. Dose levels I-III have been fully recruited. In dose level IV two patients with visceral metastasis are ongoing on study medication. Safety and clinical data will be available after the last patient discontinues study treatment.

Information on the study protocol, the translational subprotocol and details on c-kit and PDGF-receptor expression analysis will be presented at the meeting.

OT3-01-03
Kalinsky K, Sparano JA, Kim M, Crew KD, Maurer MA, Taback B, Feldman SM, Hibshoosh H, Wiechmann LS, Adelson KB, Hershman DL, Columbia University Medical Center, New York, NY; Albert Einstein College of Medicine, New York, NY; Mount Sinai School of Medicine, New York, NY

Background: To serve as a bridge between pre-clinical and early clinical testing, pre-surgical studies are being designed to expose patients to a limited duration of an anti-cancer agent before launching a lengthy clinical trial program. In this model, modulation of predictive tissue and serum biomarkers are assessed. At Columbia University, we have completed 3 pre-surgical studies. The PI3K/Akt signaling pathway is an important signaling pathway in breast cancer (BC). The first allosteric Akt inhibitor to enter clinical development, MK-2206 is well-tolerated and has demonstrated anti-cancer properties in pre-clinical and early-phase clinical studies. The purpose of this study (NCI P8740) is to determine the effects of MK-2206 on women with newly diagnosed BC between breast biopsy and surgery. Trial Design: Patients (n=30) will receive 2 doses of weekly MK-2206 (200 mg): first dose at day -9 and second at day -2 from surgery. This drug administration consistency will decrease the potential for noise in detecting true biomarker response. This time period was selected based upon the pharmacokinetic profile of weekly MK-2206. The main eligibility criteria for this open-label pre-surgical trial include operable, clinical stage I (at least T1c) to IIIC invasive BC. Patients not considered for neoadjuvant chemotherapy are eligible. Specific Aims: Our hypothesis is that MK-2206 will decrease phospho-AktSer473 in tumor tissue after 2 weekly doses. To maintain sample quality and ensure that phospho-Akt evaluated on formalin-fixed paraffin-embedded (FFPE) tissue is comparable across samples (given the 20 minute half life of AKT), all samples will be processed rapidly by a standardized protocol. Secondary objectives include the following in FFPE: modulation of downstream PI3K/Akt pathway signaling [immunohistochemistry and reverse phase-protein microarray analysis (RPMA)]; change in tumor proliferation (Ki-67 staining); and exploration of whether PI3K/Akt signaling change depends upon tumor genetics (PIK3CA mutation or PTEN loss) or expression (Hormone Receptor/HER2 status). Pre- and post-MK-2206 blood will be collected for phospho-expression analysis in peripheral blood mononuclear cells (RPMA and western blotting). Statistical Methods: With the expectation that 20% of the matched pre- and post-treatment tissue samples will not be analyzable, we will enroll 30 patients to ensure that matched tumor samples from 24 patients are available for our primary analysis. A sample size of 24 patients will yield greater than 90% power to detect a difference in the mean score between pre-and-post treatment samples of 60 units, paired t-test (2-sided, 0.05 significance level). This trial was recently activated by the NCI and currently ready to accrue patients. Patients will be enrolled and treated at affiliated institutions in the New York Cancer Consortium.
OT3-01-04

TANIA: A Randomized Phase III Trial Evaluating Continuing and Reinstituted Bevacizumab (BEV) in Patients Previously Treated with 1st-Line BEV for Locally Recurrent/Metastatic Breast Cancer (LR/mBC).

von Minckwitz G, Cortés J, Gligorov J, Marschner NW, Puglisi F, Vrdoljak E, Duene A-A, Zielinski C. German Breast Group, GGB Forschungs GmbH, Neu-Isenburg, Germany; Vall d’Hebron University Hospital, Barcelona, Spain; APHP Tenon-APREC, Paris, France; Outpatient Cancer Centre, Freiburg, Germany; University Hospital of Udine, Udine, Italy; Centre of Oncology, Split, Croatia; F. Hoffmann-La Roche Ltd, Basel, Switzerland; Medical University of Vienna, Vienna, Austria

Background: BEV has repeatedly demonstrated improvements in progression-free survival (PFS) in randomized phase III trials, providing benefit in the 1st- and 2nd-line settings when combined with chemotherapy versus chemotherapy alone. However, the role of BEV in BEV-pretreated LR/mBC is unclear. The TANIA trial (MO22998) was initiated to address this question.

Trial design: TANIA is an open-label randomized multicenter phase III trial. Eligible patients are aged ≥18 years with HER2-negative LR/mBC that has progressed during or after ≥12 weeks of 1st-line BEV combined with chemotherapy for LR/mBC. Maintenance therapy with BEV and/or endocrine therapy before study enrollment is permitted. Patients who have previously received anti-angiogenic therapy other than BEV in the 1st-line LR/mBC setting are not eligible. BEV-specific exclusion criteria are similar to previous randomized trials (e.g. inadequately controlled hypertension; history of nephrotic syndrome, hypertensive crisis, gastrointestinal perforation, or grade ≥3 venous thromboembolism; significant vascular disease). All patients must provide written informed consent. After stratification according to hormone receptor status, time to 1st progression (<6 vs ≥6 months), choice of chemotherapy (taxane vs non-taxane vs vinorelbine), and LDH level (≤1.5 vs >1.5 × upper normal limit), eligible patients are randomized 1:1 to 2nd-line treatment with either standard single-agent chemotherapy alone (arm 1) or standard single-agent chemotherapy combined with BEV (15 mg/kg q3w or 10 mg/kg q2w, depending on the chosen chemotherapy regimen; arm 2). 2nd-line therapy is continued until disease progression, unacceptable toxicity, or patient withdrawal. At progression, patients in arm 1 receive 3rd-line chemotherapy without BEV (ie no crossover permitted) and those in arm 2 receive 3rd-line chemotherapy in combination with BEV (unless prevented by unacceptable toxicity). Maintenance endocrine therapy is permitted in both arms (in combination with BEV in arm 2). At 3rd progression, BEV is permitted in all patients. The primary objective is to determine the therapeutic benefit of continued or reintroduced BEV in combination with 2nd-line chemotherapy for patients previously treated with 1st-line BEV plus chemotherapy, determined by the duration of PFS from the time of randomization to 2nd progression (or death). Additional objectives include evaluation of the interval between 2nd and 3rd progression, the interval between randomization and 3rd progression, PFS in stratified subgroups, overall response rate to 2nd-line therapy, overall survival (OS), 1-year OS rate, safety, quality of life (FACT-B and EQ-5D), and translational research. The sample size of 488 patients is calculated assuming median PFS of 7 months in arm 1, a hazard ratio of 0.75, a recruitment period of 30 months, and a 5% dropout rate (ie 439 eligible patients), providing ≥80% power at two-sided α=0.05. As of 15th June 2011, 23 patients have been enrolled. The primary analysis is planned after 384 patients have shown progression on 2nd-line therapy.

OT3-01-05

PARP Inhibition after Preoperative Chemotherapy in Patients with Triple-Negative Breast Cancer (TNBC) or Known BRCA 1/2 Mutations: Hoosier Oncology Group BRE09-146.

Miller KD, Perkins SM, Badve SS, Sledge GW, Schneider BP. Indiana University Melvin and Bren Simon Cancer Center

Background: Based on recently reported I-SPY trial, TNBC patients who had residual disease category II or III had 2-year disease free survival (%) of only ~40% (J Clin Oncol 2009;27:18s). Currently, no standard systemic therapy exists for this high-risk group. It represents a real opportunity to explore the potential impact of novel therapies. Recent laboratory and early clinical studies (Nature 2005;434:913) identified a unique sensitivity to DNA-damaging chemotherapy and PARP inhibition. We initiated a randomized phase II trial of DNA-damaging chemotherapy (cisplatin) or PARP-inhibition + cisplatin in TNBC patients with substantial residual invasive disease after standard anthracycline and/or taxane containing neoadjuvant chemotherapy. Methods: To ensure a high-risk population, patients must have residual disease category 0-2 based on the Miller-Payne classification system, residual cancer burden classification II or III, residual lymph node involvement, or at least 2 cm of residual invasive disease in the breast. After completion of standard radiation therapy (when indicated), patients are randomized 1:1 to cisplatin (75 mg/m2 IV Day 1 every 3 weeks x 4 cycles) alone or in combination with PARP inhibition (PF-01367338 - 24 mg IV D1, 2, 3 of each 3 week cycle with a single dose escalation to 30 mg in the absence of significant toxicity in cycle 1 followed by maintenance PARP inhibition weekly x 24 weeks). The primary objective is 2-year DFS. To detect an improvement in 2-year DFS from 40% with cisplatin alone to 63.2% in the cisplatin + PF-01367338 arm (corresponding to HR=0.5), with 80% power using a one-side log-rank test with 0.10 level of significance, 102 patients are required in the primary analysis. Secondary objectives include safety, 1-year DFS, overall survival, and biomarkers of tumor recurrence, resistance to chemotherapy and/or PARP inhibition. Two dose escalation safety cohorts (N=13) were completed without dose limiting toxicity; the randomized portion began enrolment in 11/2010 has enrolled 20 patients as of 05/2011.

OT3-01-06

A Phase 2 Study Investigating the Safety, Efficacy and Surrogate Biomarkers of Response of 5-Azacitidine (5-AZA) and Entinostat (MS-275) in Patients with Advanced Breast Cancer.

Connor RM, Jankowitz RC, Andreopoulos E, Alred JB, Jeter SC, Zorzi J, Adam BM, Espinoza-Delgado I, Baylin SB, Zahnow CA, Ahuja N, Davidson NE, Stearns V, Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins University, Baltimore, MD; University of Pittsburgh Cancer Institute, Pittsburgh, PA; Albert Einstein College of Medicine, Montefiore Medical Center, New York, NY; Mayo Clinic, Rochester, MN; National Cancer Institute, Bethesda, MD

Background: Epigenetic alterations in the genome, including abnormal DNA methylation and histone hypoacetylation, initiate and promote cancerous changes via several mechanisms, including inactivation of tumor suppressor genes. Preclinical investigations in breast cancer suggest that use of epigenetic modifiers results in re-expression of aberrantly silenced genes and proteins that represent important therapeutic targets (e.g. estrogen receptor alpha, ER). Combination therapy with a DNA methyltransferase inhibitor (DNMTI) and a histone deacetylase inhibitor (HDACI) has yielded superior ER re-
expression and greater restoration of tamoxifen responsiveness than with HDACI alone. We hypothesize that clinically tolerable doses of the DNMTI 5-azacitidine (5-AZA) and the HDACI entinostat may not only effect changes in DNA methylation and gene expression, but also yield objective disease responses in women with advanced breast cancer. 

Trial design: 
This multicenter phase II study (NCT01349959) is enrolling patients with advanced human epidermal growth factor receptor 2 (HER2)-negative breast cancer with triple negative (ER/PR/HER2-negative, Cohort A) or hormone-resistant (Cohort B) disease. Patients will receive 5-AZA 40 mg/m2 subcutaneously days 1-5 and 8-10 and entinostat 7 mg orally days 3 and 10 every 28 days. Because of the potential for re-expression of the ER with epigenetic agents, patients will be offered continuation of 5-AZA and entinostat at progression with the addition of hormonal therapy (investigator discretion). Mandatory tumor biopsies will be performed at baseline and after 8 weeks of therapy to evaluate correlative biomarkers. 

Eligibility Criteria: 
Eligible patients must be ≥ 18 years, have measurable locally advanced/metastatic triple-negative (at least one prior chemotherapy received adjuvant/metastatic setting) or hormone-resistant (must have received two prior hormonal agents and one prior chemotherapy) disease, adequate organ function and ECOG PS ≤ 2. 

Specific Aims: 
1. Objective response rate (ORR) by RECIST 1.1 criteria. 
2. Safety and tolerability 
3. Progression-free survival, overall survival and clinical benefit rate. 
4. Safety and toxicity data, feasibility and response rate where hormonal therapy is added to the combination under investigation at the time of progressive disease. 
5. Pharmacokinetics, cytidine deaminase, changes from baseline of candidate gene methylation and expression in circulating deoxyribonucleic acid (DNA) and malignant tissue. 

Statistical Methods: 
Using a two-stage three-outcome design to assess the efficacy of the combination, a maximum of 30 patients (requiring 27 evaluable) will be accrued to each cohort unless undue toxicity is encountered for a maximum sample size of 60 patients. The study design tests the null hypothesis that the ORR is at most 5% against the alternative hypothesis that it is at least 20% with a type I error of 4% and power of 90%. Present and Targeted Accrual: This study has just opened to patient enrollment. We anticipate a rapid accrual of 60 patients within 1 year. 

Funding from Stand Up to Cancer and CTEP). 

OT3-01-07 
The BEACON Study (BrEAst Cancer Outcomes with NKTR-102): A Phase 3 Open-Label, Randomized, Multicenter Study of NKTR-102 Versus Treatment of Physician’s Choice (TPC) in Patients (pts) with Locally Recurrent or Metastatic Breast Cancer (MBC) Previously Treated with an Anthracycline, a Taxane, and Capecitabine (ATC). 

Awada A, Leung ACF, Zhao C, Hannah AL, Perez EA. Universite Libre de Bruxelles, Brussels, Belgium; Nektar Therapeutics, San Francisco, CA; Mayo Clinic, Jacksonville, FL 

Background: NKTR-102 is a next-generation topoisomerase I inhibitor-polymer conjugate with a markedly reduced Cmax and a continuous exposure profile compared to irinotecan. A phase 2 trial of single-agent NKTR-102 compared a dose of 145 mg/m2 every 2w or every 3w in 3rd-line MBC (Awada et al, ASCO 2011). Overall the ORR was 29% (including 3% CR) with the prior ATC subset demonstrating an ORR of 31%. Dosing every 3w was better tolerated; in this arm, median PFS equaled 5.3m and median OS equaled 13.1m. 

Trial Design: NKTR-102 will be compared to TPC in an open-label, randomized, parallel, two arm multicenter Phase 3 pivotal study in pts with previously treated locally recurrent or metastatic breast cancer. 

Key Entry Criteria: Adult females, with ECOG 0 or 1 with adequate liver, kidney and marrow function. All patients must have received prior therapy with an anthracycline (in neo/adjuvant or metastatic setting or both), a taxane (in neo/adjuvant or metastatic setting or both) and capecitabine (in neo/adjuvant or locally advanced/metastatic setting or both) unless not medically appropriate or explicitly contraindicated for the patient. All chemotherapy- and radiation-related toxicities must have resolved to ≤ Grade 1, except for stable sensory neuropathy ≤ Grade 2 and alopecia. 

Funding from Stand Up to Cancer and CTEP). 

OT3-01-08 
Phase II Study of S-1 Combined with Cisplatin in the First-Line Treatment of Triple Negative Breast Cancer. 

Fan Y, Xu BH. Cancer Hospital & Institute, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, China 

Rationale: There is no standard choice of first-line treatment for triple negative breast cancer (TNBC). It is supposed that TNBC may be more sensitive to DNA damage agents such as platinum but resistant to cell cycle specific agents including taxanes and 5-fluorouracil. Currently these hypotheses are under investigation in several ongoing clinical trials. S-1 is an orally administered fluorinated pyrimidine with high activity in non-small-cell lung cancer and gastric cancer. Recently single agent S-1 has been reported to be effective in capecitabine-resistant metastatic breast cancer with a response rate of 27.8% and median TTP of 6.2 months. So it is intriguing to find whether S-1 plus cisplatin would be a valuable regimen in TNBC patients with limited choices of management. 

Design: This is a prospective, single-arm phase II study. Eligible patients are treated first with S-1 (80mg/m2, 2 weeks on and 1 week off) and cisplatin (75mg/m2 intravenously day1) every 3 weeks as a cycle for up to 6 cycles, until disease progression, unacceptable toxicity or patient consent withdrawal. If no PD was observed after 6 cycles, patients can remain on S-1 until disease progression, unacceptable toxicity or patient consent withdrawal. 

Eligibility: Major inclusion criteria includes: 1. histologically confirmed triple negative breast cancer; 2. advanced patients without
prior treatment for locally recurrent or metastatic disease; 3. patients must have received anthracyclines and taxanes in neo/adjuvant setting; 4. at least one measurable disease according to RECIST 1.1; 5. adequate organ function within acceptable range.

Endpoints: The primary endpoint is objective response rate (ORR) and secondary endpoints include progression free survival (PFS), overall survival (OS) and safety profile. Exploratory biomarks will be studied for TS and DPD enzyme expression and genotyping.

Statistical considerations: The expected response rate is around 50% based on the literature, and the null hypothesis is based on a response rate no greater than 30%. According to Simon’s two stage phase II design with errors of 0.05 and 0.20 respectively, a minimum of 15 patients would be required in the first stage and totally 46 patients after the second stage. Since a non-compliance rate of 10% is expected, we plan to enroll a total of 50 patients.

Recruitment: Currently it has just been initiated with 1 patient still on screening. The target accrual, as stated above, is 50 patients.

OT3-01-09
Phase 3 Trial Comparing Capericitabine in Combination with Sorafenib or Placebo for Treatment of Locally Advanced or Metastatic HER2-Negative Breast Cancer (RESILIENCE).

Baselga J, Schwartzberg LS, Petrenciu O, Shan M, Gradihar WJ. Massachusetts General Hospital Cancer Center; West Clinic; Bayer HealthCare Pharmaceuticals; Feinberg School of Medicine, Northwestern University

Sorafenib (SOR) is an oral multikinase inhibitor with antiangiogenic and antiproliferative activity. SOR is currently indicated for renal cell and hepatocellular carcinoma, with indications in other tumor types being explored. In a double-blind, randomized phase 2b screening trial (SOLT-0701) in patients with advanced HER2-negative breast cancer (BC), the addition of SOR to capecitabine (CAP) showed a statistically significant improvement in the primary endpoint of progression-free survival (PFS) compared with placebo (PL)+CAP (median 6.4 vs 4.1 mo; hazard ratio=0.58; 1-sided P=0.0006). The combination was tolerable. Grade 3/4 adverse events were comparable between treatment arms with the exception of grade 3 hand-foot skin reaction/syndrome (HFSR/HFS) (44% in SOR+CAP vs 14% in PL+CAP). The SOLT-0701 results indicate a potential role for the oral combination of SOR+CAP in the treatment of BC and support a phase 3 trial.

Design: RESILIENCE is an ongoing multinational, double-blind, PL-controlled, randomized phase 3 trial designed to assess SOR+CAP as a first- or second-line therapy in advanced HER2-negative BC. Eligibility criteria include: ≥18 years of age; ≤1 prior chemotherapy regimen for advanced BC; and resistant to/failed taxane and anthracycline or no indication for further anthracycline treatment. Prior hormonal or radiation therapy is allowed, but prior use of VEGF inhibitors is not. Patients with significant cardiovascular disease or active brain metastases are not eligible. Patients are stratified by hormone receptor status, geographic region, and prior chemotherapy for advanced BC and randomized (1:1) to CAP (1000 mg/m² po twice daily [BID], days 1–14 of a 21-day cycle) in combination with SOR (po BID, days 1–21, total dose 600 mg/day) or matching PL. CAP and SOR/PL doses can be escalated to 2500 mg/m² per day and 800 mg/day, respectively, as tolerated. The protocol outlines strategies to manage toxicities with dose interruption and reduction. Dose re-escalation after reduction is allowed for SOR/PL (per protocol guidance) but not for capecitabine. Guidelines are provided for prophylactic and symptomatic treatment of HFSR/HFS. Radiographic assessment is every 6 wk for the first 36 wk, and every 9 wk thereafter.

The primary endpoint is PFS. Assuming a 1-sided alpha of 0.005 and a power of 98.9%, the sample size is estimated at ~519 patients, with primary analysis planned after 363 events. Secondary endpoints include overall survival, time to progression, overall response rate (RECIST 1.1 criteria), duration of response, and safety. In addition, patient reported outcomes will be assessed, and the trial will include an exploratory analysis of biomarkers. Enrollment began in Nov 2010. The trial is registered at ClinicalTrials.gov (NCT01234337).

Conclusions: RESILIENCE will provide definitive PFS data for SOR+CAP as a first- or second-line therapy in HER2-negative locally advanced or metastatic BC. The phase 3 design and improved dosing guidance since SOLT-0701 will better characterize the benefit-to-risk profile of this regimen.

OT3-01-10
Withdrawn by Author

OT3-01-11
A Randomized, Phase II Multicenter, Double-Blind, Placebo-Controlled Trial Evaluating MetMAb and/or Bevacizumab in Combination with Weekly Paclitaxel in Patients with Metastatic Triple-Negative Breast Cancer.

Daniel BR, Campone M, Dieras V, Ervin T, Yu W, Paton VE, Xia Q, Peterson A. Chattanooga Oncology Hematology Associates - SCRI, Chattanooga, TN; Centre Rene Gauducheau, Nantes-Saint Herblain, France; Institute Curie, Paris, France; Florida Cancer Specialists, Englewood, FL; Genentech Inc., South San Francisco, CA

Background: Dysregulation of the HGF/Met pathway has been associated with tumorigenesis in many malignancies, including the basal sub-type of triple-negative breast cancer. MetMAb (RG3638) is a recombinant, humanized, monovalent monoclonal antibody directed against Met. By binding to the extracellular domain of Met, MetMAb selectively blocks ligand binding and subsequent activation by HGF. Pre-clinical data support the efficacy of combining MetMAb with numerous chemotherapy agents and with targeted agents including bevacizumab and erlotinib. In clinic, MetMAb has been generally well tolerated as a single agent (Phase I), in combination with bevacizumab (Phase Ib) and with bevacizumab in a dose escalation/expansion study (Phase Ib') as well as in combination with erlotinib in patients with previously treated NSCLC. The combination of MetMAb + erlotinib in NSCLC demonstrated significant benefit in both PFS and OS in patients with Met diagnostic positive tumors whereas those patients with Met diagnostic negative tumors demonstrated a detrimental effect in both PFS and OS. The most commonly reported adverse events associated with MetMAb are peripheral edema and fatigue.

Methods: This clinical trial is a randomized three-arm Phase II study in patients with triple-negative metastatic breast cancer, which makes up the majority of basal sub-type breast cancer. Patients will be randomized (1:1:1) to either paclitaxel + bevacizumab + placebo; paclitaxel + placebo + MetMAb; or paclitaxel + bevacizumab + MetMAb. The primary endpoint of this study is PFS in all patients and by Met diagnostic status. Secondary endpoints include an evaluation of OS, ORR, safety, and pharmacokinetics. To date, 11 patients have been enrolled, and 10 patients have been treated.

Primary and secondary analyses will include all randomized patients, with patients analyzed according to the treatment arm to which they were assigned. Kaplan-Meier methodology will be used to estimate the median PFS for each treatment arm. An estimate of the HR with 95% CI will be determined using a Cox regression model with an
indicator variable for the MetMAb-containing arm. Safety will be assessed through summaries of adverse events and will include all patients who receive any amount of study treatment. This study remains open for accrual; further details on the trial can be found on the ClinicalTrials.gov website under NCT01186991.


OT3-01-12
Withdrawn by Author

OT3-01-13
Phase One Trial of Combined Temsirolimus, Erlotinib, and Cisplatin in Advanced Solid Tumors.


Background: A large subset of triple negative breast cancers (TNBC) are driven in part by a combination of activated growth factor signaling and downstream constitutive activation of the PI3K pathway. One therapeutic strategy is to target each driver simultaneously, requiring an understanding of drug interactions and combined toxicities. There is a strong correlation between loss of PTEN and both expression of EGFR and mutation of p53 in a subset of TNBC. Single agent trials targeting EGFR in breast cancer have been a disappointment likely, in part, due to constitutive downstream activation of PI3K, AKT and mTOR signaling, mainly through loss of PTEN expression. Preclinical studies have demonstrated 1) that resistance to EGFR-targeted therapy in the setting of an activated PI3K pathway can be overcome with rapamycin (which inhibits the TORC1 mTOR complex), and 2) that a subset of TNBC cells with p53 mutations are particularly sensitive to the DNA damaging agent cisplatin due to their high expression of p63 and p73. We have hypothesized that targeting the EGFR, PTEN and DNA damage pathways simultaneously with the rapalog temsirolimus, the EGFR inhibitor erlotinib, and cisplatin will provide therapeutic benefit in a definable subset of TNBC patients.

Design: Single institution phase one dose escalation trial of combined temsirolimus, erlotinib, and cisplatin in advanced solid tumor patients. Cisplatin and temsirolimus are given on day 1 and day 8 of 21 day cycles and erlotinib is taken daily without interruption. Patients are dose escalated using a standard 3+3 design. Cisplatin is given at a fixed dose of 30 mg/m². Erlotinib was started at 100 mg and will be escalated to 150 mg. Temsirolimus was started at 15 mg, and after erlotinib escalation, will be escalated to 25 mg. The objectives of the study are: 1) characterize toxicity of the regimen and establish combined maximal tolerated dosing, 2) measure drug target inhibition by assessing EGFR, AKT, and mTOR activation in serial assessments of peripheral blood mononuclear cells and skin biopsies, 3) measure the pharmacokinetics of all three drugs in combination, and 4) exploratory assessment of molecular measures of PTEN, EGFR, and p53 status, and markers of activated downstream pathways in patient’s archived pathology specimens to assess feasibility of identifying candidate biomarkers in future trials involving patients with TNBC. Eligibility criteria include one prior treatment for advanced disease, ECOG PS=0-1, measurable or non-measurable disease, available archived tumor sample, non-smoking, normal renal function, and lack of concurrent use of strong CYP3A4 inhibitors or inducers. Because of significant rash encountered in prior combination trials of EGFR and TORC1 inhibitors, a prophylactic oral doxycycline strategy supported by randomized trials is being employed. Current accrual is 6 out of a possible 18 patients.

OT3-01-14
N0937: Phase II Trial of Brostallicin and Cisplatin in Patients with Metastatic Triple Negative Breast Cancer.

Moreno-Aspitia A, Rowland KM, Liu H, Hillman DW, Stella PJ, Perez EA. Mayo Clinic, Jacksonville, FL; Mayo Clinic, Rochester, MN; Carle Cancer Center, Urbana, IL; St. Joseph Mercy Cancer Center, Ypsilanti, MI

Background: Tumors that are negative for estrogen and progesterone receptors and do not over express HER2 are referred as “triple negative” breast cancer (TNBC). These tumors are characterized by unique molecular profiles on gene expression arrays, aggressive behavior with a high recurrence rate, an increased risk of visceral metastases, poor prognosis and lack of targeted therapies. Brostallicin is a novel synthetic compound from the class of DNA minor groove binding (MGB) anti-cancer agents. It retains activity in cancer cells resistant to alkylating agents, topoisomerase I inhibitors and is fully active against DNA-mismatch repair deficient tumor cells. Cells expressing relatively high glutathione/glutathione S-transferase (GSH/GST) levels are more susceptible to brostallicin antitumor efficacy. Cisplatin administration increases expression of GST in tumor cells leading to an increased anti-tumor efficacy of brostallicin.

Trial design: Single-stage phase II study - based on the effects of cisplatin on GSH/GST levels in preclinical models, the most reasonable sequence to explore was cisplatin on Day 1 followed by brostallicin on Day 2 repeated every 21 days.

Eligibility criteria: Women or men ≥18 years of age with confirmed adenocarcinoma of the breast with clinical evidence of measurable metastatic disease and triple negative subtype according to current ASCO CAP guidelines [ER/PR ≤1%; HER2 negative], who received 0-4 prior chemotherapy regimens in the metastatic setting; with adequate hematologic, renal and hepatic functions; and no active CNS metastases.

Aims: To study the efficacy of the novel drug, brostallicin, as well as to serve as proof of concept of its mechanism of action in TNBC. The primary endpoint is to evaluate clinical efficacy of the combination of brostallicin and cisplatin in the treatment of patients with metastatic TNBC, as measured by progression-free survival (PFS) at 3 months with 89% power (0.10 significance level) to detect an absolute difference of 20%. Secondary endpoints include ORR by RECIST, duration of response, 6-month PFS, overall survival (OS) and adverse event profile. Tertiary endpoints include assessment of 1) GSH levels prior to the administration of cisplatin and of brostallicin; and 2) the prevalence of BCRA-1 mutation by IHC in the primary or metastatic tumor.

Statistical methods: The largest 3-month PFS proportion where the proposed treatment regimen would be considered ineffective in this population was estimated at 35% based on the median PFS of 60 days in patients with metastatic TNBC enrolled in the N0234 trial (erlotinib and gemcitabine as 1/2nd line), and the smallest 3-month PFS success proportion that may warrant subsequent studies with the proposed regimen in this patient population was estimated at 55%. The interim analysis will be reported when the 20th eligible patient has been followed for 3 months.

Present accrual and target accrual: 21 patients have been accrued at the time of abstract submission (June 2011). Target accrual is 42 evaluable patients.
**OT3-01-15**

**Phase III Trial Evaluating the Addition of Bevacizumab to Endocrine Therapy as First-Line Treatment for Advanced Breast Cancer: The LEA Study.**

*De la Haba-Rodriguez JR, von Minckwitz G, Martin M, Morales S, Crespo C, Guerrero A, Anton-Torres A, Gil M, Muñoz M, Carrasco E, Rodríguez-Martin C, Porras I, Aktas B, Schoeneegg W, Tio J, Mehta K, Loibl S, On Behalf of GEICAM and GBG. University Reina Sofia Hospital, Córdoba, Spain; German Breast Group, Neus-Isenberg, Germany; Universitario Gregorio Marañon, Spain; Hospital Arzum de Vilanova de Lleida, Spain; Hospital U. Ciudad de Murcia, Spain; Hospital University Hospital, Spain; Hospital Universitario Ramón y Cajal, Spain; Instituto Valenciano de Oncologia, Spain; Miguel Servet University Hospital, Spain; Institut Catala d’Oncologia, Spain; Provincial Hospital Clinic, Barcelona, Spain; GEICAM Headquarters, Madrid, Spain; University Hospital, Essen, Germany; Praxis Dr Schnoenegg, Berlin, Germany; Universitätsklinikum Muenster, Germany*

**Background:** Retrospective clinical data suggest that high vascular endothelial growth factor (VEGF) levels in breast tumors are associated with a decreased response to endocrine therapy. We designed the randomized phase III LEA study of first-line bevacizumab in combination with endocrine therapy, to address the hypothesis that anti-VEGF treatment can prevent resistance to endocrine therapy in patients with advanced breast cancer sensitive to such treatment.

**Methods:** Postmenopausal patients with evaluable locally recurrent or metastatic breast cancer, HER2-negative and estrogen receptor (ER)- and/or progesterone receptor (PgR)-positive disease, and eligible to receive hormonal treatment are candidates for this study. Patients are randomized to receive letrozole 2.5mg daily or fulvestrant, 250mg every 4 weeks (Arm A) or the same hormonal therapy plus bevacizumab 15mg/kg every 3 weeks (Arm B). The primary objective is to compare progression-free survival (PFS) between the treatment arms. Secondary endpoints are overall survival, time to treatment failure, overall response rate, response duration, clinical benefit rate and safety. In total, 344 patients (172 in each treatment arm) will be randomized to receive letrozole or fulvestrant.

**Results:** Recruitment began in November 2007. To date, 348 patients have been included in the study in Spain (n=244) and Germany (n=104). We anticipate completing recruitment by September 2011. Baseline characteristics of the first 334 randomized patients are shown in the table.

**Conclusions:** LEA is the first study to explore the use of an antiangiogenic drug in combination with endocrine therapy in the context of a phase III study.

**OT3-01-16**

**A Phase 2 Study of Ridaforolimus (RIDA) and Dalotuzumab (DALO) in Estrogen Receptor Positive (ER+) Breast Cancer.**

*Lu BD, Blum JL, Cortes J, Rugo HS, Swanton C, Eaton L, Song Y, Zhang T, Ebbinghaus SW, Baselga J, Merck Research Laboratories, Kenilworth, NJ; Texas Oncology, Dallas, TX; Vall D’Hebron University Hospital, Barcelona, Spain; UCSF Helen Diller Family Comprehensive Cancer Center, San Francisco, CA; The Royal Marsden Hospital, Sutton, United Kingdom; Merck Research Laboratories, North Wales, PA; Merck Research Laboratories, Boston, MA; Massachusetts General Hospital, Boston, MA*

**Background:** Activation of the PI3K/AKT/mTOR pathway is common in breast cancer. Preclinical studies indicate that the dual inhibition of IGFR and mTOR may be additive or synergistic and abrogate the feedback activation of AKT due to rapamycin analog mTOR inhibitors. A phase 1 study of the mTOR inhibitor RIDA and the anti-IGFR antibody DALO demonstrated that the combination was feasible and well-tolerated at doses that were nearly those used for the two single agents and with dose limiting toxicity of stomatitis, similar to RIDA as monotherapy. A preliminary signal of anti-tumor activity, including partial responses and prolonged progression free survival, was observed in ER+ breast cancer, especially in high proliferation tumors. This observation is supported by data from tumor profiling including partial responses and prolonged progression free survival, was observed in ER+ breast cancer, especially in high proliferation tumors. This observation is supported by data from tumor profiling and preclinical data suggesting that ER+ high proliferation breast cancer may be responsive to the RIDA-DALO combination. Methods: This is a two-part, adaptive design study intended to first test the combination of RIDA-DALO against a standard agent, exemestane, for ER+ positive breast cancer that has progressed after aromatase inhibitors. The trial is a multi-center, international, randomized study for ER+ positive breast cancer that has progressed after aromatase inhibitors. The trial is a multi-center, international, randomized study with PFS as the primary endpoint. Patients (pts) are stratified into high and low proliferation strata based on baseline Ki67, and the low proliferation stratum is capped to ensure adequate enrollment of high proliferation pts. If Part A shows a PFS benefit for RIDA-DALO, Part B is intended to show the PFS benefit of the combination over the single agents by comparing RIDA-DALO to RIDA and DALO. Part B will be further adapted based on whether the PFS benefit in Part A is observed in all-comers or the high proliferation subset. Tumor tissue is collected for molecular profiling and analysis of intrinsic subtype, with an efficacy analysis based on classification.
of tumors using the genomic grade index (GGI) as an exploratory objective. This trial design is intended to establish proof of concept that RIDA-DALO can prolong PFS in ER+ breast cancer in all-comers or a high proliferation subset by Ki67, while efficiently addressing the development of two investigational agents in combination and leading to a two-arm Phase 3 design.

OT3-01-17
Randomized, Double-Blind, Placebo-Controlled Phase II Trial of Low-Dose Metronomic Cyclophosphamide Alone or in Combination with Veliparib (ABT-888) in Chemotherapy-Resistant ER and/or PR-Positive, HER2/neu-Negative Metastatic Breast Cancer: New York Cancer Consortium Trial P8853.

Andreopoulou E, Chen AP, Zujewski JA, Kalinsky K, Vahdat L, Raptis G, Hershman D, Novic Y, Muggia F, Sparano J. Albert Einstein College of Medicine/Montefiore Medical Center, Bronx, NY; National Cancer Institute, Bethesda, MD; Columbia University Medical Center, New York, NY; Weill Cornell Medical College, New York, NY; Mount Sinai School of Medicine, New York, NY; New York University Langone Medical Center, New York, NY

Background: Veliparib is an orally available, small molecule inhibitor of poly(ADP-ribose) polymerase (PARP). PARP is an essential nuclear enzyme that plays a role in recognition of DNA damage and facilitation of DNA repair. PARP inhibitors potentiate the cytotoxicity of DNA-damaging agents, including cyclophosphamide (C). The rationale for the proposed trial is as follows: (1) low-dose, continuous metronomic C (50 mg PO daily) has activity in refractory metastatic breast cancer (MBC), (2) PARP is induced by DNA damaging agents, (3) PARP expression is comparable in ER-positive and ER-negative disease, (4) some ER-positive breast cancers exhibit defective homologous recombination pathway repair genes (eg, RAD51 and XRCC3), (5) the PARP inhibitor iniparib appears to be more effective when used in chemotherapy resistant disease. Taken together, these findings suggest that veliparib-C combination may be more effective than metronomic C alone in chemotherapy resistant MBC.

Trial design: A randomized Phase II trial design 1:1: S. Blocked randomization will be performed at all participating sites. Patients are randomized to oral C (50mg PO daily) plus either veliparib (60mg PO daily) or matching placebo.

Eligibility criteria: (1) ER- and/or PR-positive, HER2-negative MBC, (2) ECOG PS 0-1, (3) at least 2 prior chemotherapy regimens for MBC, including a taxane and capcetabine. 4) at least 1 line of endocrine therapy for metastatic disease (includes relapse while receiving endocrine therapy).

Specific aims: Primary: To determine if the addition of veliparib to metronomic dose C improves median progression free survival (PFS) compared with C alone in patients with ER and/or PR-positive, HER2-negative MBC who progressed on at least two lines of prior chemotherapy and one line of prior endocrine therapy.

Secondary: 1) To determine if the addition veliparib to C chemotherapy improves a) response rate b) clinical benefit rate (defined as objective response plus stable disease for at least 24 weeks from day +1) 2) Survival in patients treated with C alone and C plus veliparib. 3) Adverse event profile in patients treated with C alone and C plus veliparib.

Translational: Exploratory analyses will evaluate whether the macroH2A1.1 and PARP1 expression status in archival paraffin, or veliparib-induced PARP downregulation in peripheral blood mononuclear cells, is predictive of benefit from veliparib.

Statistical methods: The primary endpoint is PFS, and the trial is powered to detect an increase in median PFS from 3 to 6 months (alpha=0.10, beta=0.10), which will require enrollment of 62 eligible patients over 12 months.

Enrollment: The study is active and open to enrollment.

Clinical trials.gov NCT01351909

OT3-01-18
Combination Immunotherapy with Trastuzumab and the HER2 Vaccine E75 in Low and Intermediate HER2-Expressing Breast Cancer Patients To Prevent Recurrence.

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Background: In a phase II trial, the HER2-derived E75 vaccine administered with the immunoadjuvant GM-CSF has been shown to reduce breast cancer recurrence in the adjuvant setting, with a greater benefit seen in patients with low levels of HER2 expression (IHC 1+ or 2+). There has also been recent suggestion that patients with low HER2 expression may also benefit from trastuzumab. Preclinical testing of the combination of trastuzumab and the E75 vaccine has shown a synergism with combinational therapy. Finally, from our phase II trials of cytotoxic T cell-eliciting peptide vaccines, sequential treatment with trastuzumab and HER2 vaccination has resulted in no recurrences in 30 patients with a median follow-up of 48 months. Based on these results, we have designed a trial to evaluate the ability of the combination of trastuzumab and the E75 vaccine to prevent breast cancer recurrence.

Trial Design: This study will be a multi-center, prospective, randomized, single-blinded, phase II trial evaluating trastuzumab + E75 + GM-CSF (immunoadjuvant) vs. trastuzumab + GM-CSF alone (no E75) in the adjuvant setting in breast cancer patients. Eligible patients include node positive (or node negative if negative for both ER and PR) disease-free breast cancer patients with low or intermediate levels of HER2 expression (IHC 1+ or 2+) and adequate cardiac function (LVEF >50%). Patients must be HLA-A2/A3 restricted. Patients will be enrolled after completing standard of care multi-modal therapy and randomized between the two treatment arms with stratification by HER2 expression (1+ or 2+) and nodal status (N0, N1, N2, or N3). Vaccinations (E75+GM-CSF or GM-CSF alone) will be administered as six monthly intradermal inoculations concurrently with trastuzumab therapy. The primary efficacy endpoint is to compare disease-free survival (DFS) between treatment arms at 24 months. Secondary Objectives will include evaluation of cardiac toxicity from combination therapy (periodic cardiac assessment with MUGA or ECHO), DFS at 36 months, and immunologic responses to vaccination. From previously published experience with trastuzumab, we expect a recurrence rate of 15% in trastuzumab (plus GM-CSF) treated patients and anticipate that the combination of trastuzumab with E75+GM-CSF will reduce this recurrence rate to 5%. In order to show a statistical difference between these recurrence rates, we plan to enroll 150 patients per treatment arm (300 total) with a type I error rate of 5% and 80% power to detect the primary endpoint. Trial accrual is anticipated to begin in January 2012, with a two year period of enrollment followed by a three year follow-up period.

Conclusion: We hypothesize that combination adjuvant immunotherapy with trastuzumab and E75 vaccination will result in a greater reduction in breast cancer recurrence than trastuzumab therapy alone and have designed a multi-center, prospective, randomized, single-blinded, phase II trial evaluating the efficacy of
this immunotherapy combination. Contact Information: This trial is sponsored by Genentech and RXi Pharmaceuticals through the Henry M. Jackson Foundation.

OT3-01-19
Phase II Study of Topical Imiquimod and Weekly Abraxane for the Treatment of Breast Cancer Cutaneous Metastases.
Higgins DM, Childs J, Parker S, Disis ML, Salazar LG. University of Washington, Seattle, WA

Background: Breast cancer (BC) cutaneous lesions present as local chest wall recurrence or as isolated sites of metastatic disease. The treatment of cutaneous lesions is challenging and includes chest wall resection, local radiation therapy, and/or salvage chemotherapy which is not curative, associated with significant morbidity, and results in overall response rates of 20-30%. Thus, investigation of novel treatment strategies is warranted. This study incorporates multimodality treatment with topical imiquimod, a TLR-7 agonist which generates an immune signal similar to that of pathogenic bacteria and Abraxane, a conventional systemic chemotherapy with potential immunostimulatory effects. Combined, these two agents provide local and systemic strategies which are potentially synergistic; and more effective than as single-agents in treating and controlling cutaneous disease.

Trial design: A Phase II single arm, non-randomized study. Patients will be sequentially enrolled and receive a maximum of 3 treatment cycles. A treatment cycle consists of topical imiquimod daily to target lesions for 4 days/week for 4 weeks in addition to Abraxane on Days 1, 8, and 15 every 28 days. Toxicity will be evaluated weekly during treatment then monthly for four months. Defined lesions are assessed at baseline and monthly. Skin biopsies are obtained pre and post treatment for histologic analysis and RT-PCR analysis of a 7 IFN-related gene signature previously associated with tumor inhibition. Immunity to BC antigens and serum TGF-β levels are also evaluated.

Aims: To evaluate the safety and anti-tumor effects of chemoimmunotherapy with topical imiquimod and Abraxane.

Eligibility criteria: Patients with progressive or relapsed BC after standard therapy who 1) have measurable cutaneous metastatic lesions, 2) are at least 7 days from last chemotherapy, 30 days from local radiotherapy and/or systemic steroids, 3) have adequate blood counts and 4) no history of active autoimmune disease. Bisphosphonates, trastuzumab, and/or hormonal therapy is allowed.

Statistical methods: Antitumor activity of target lesions will be assessed per modified WHO criteria. Complete response (CR)-complete clearance of lesions; Partial response (PR) ≥ 50% decrease in lesion size; Stable disease (SD) < 50% decrease in lesion size; Progressive disease (PD) increase in ≥ 25% lesion size. Historical overall response rates (ORR) with second and third line salvage chemotherapy range from 20-30%, with CR rates less than 2%. Based on these numbers, an ORR of 50% or a CR rate of 10% will be used as benchmarks for success (i.e., ≥ 8 responses or ≥ 2 CRs among 15 patients (observed ORR of ≥ 53% or observed CR rate of ≥ 13%) to consider the treatment worthy of further study. As a measure of the precision of the estimate of ORR achievable with 15 patients, if the response rate is 60%, we will be 80% confident that the observed RR is within 0.16 of the true RR with 15 patients treated. Toxicity will be evaluated by CTC/3E v. 3.0 and descriptive statistics will be used to summarize changes from baseline and for reporting of immunological parameters.

Accrual: 10 patients received treatment with a target accrual of 15.

OT3-02-01
A 1-Year Prospective Longitudinal Study of the Role of Psychosocial Factors in Adherence to Adjuvant Endocrine Therapy in Early Breast Cancer.
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Background: Extensive evidence suggests that endocrine therapy (ET) and adherence to adjuvant ET directly impacts on disease-free and overall survival in women with hormone receptor positive (HR+) early stage breast cancer (EBC). Despite this, adherence to ET is suboptimal, with 35%-50% of patients discontinuing therapy earlier than recommended. Interventions to improve adherence are lacking, due in part to a poor understanding of the modifiable risk factors influencing adherence. Virtually all authors have focused on educational needs of patients and side effect management of ET but there remains a paucity of data on how potentially modifiable psychosocial factors present at outset of treatment initiation influence adherence. We believe that a better understanding of these factors may lead to interventions which enable women to adhere to long term life-saving therapies. This study aims to identify such factors guided by the Common-Sense Model of Self-Regulation which proposes that cognitive, emotional, coping and interpersonal factors are modifiable determinants of adherence.

Study Design: The study is a single centre prospective longitudinal quantitative study. Women diagnosed with HR+ EBC for which endocrine therapy is intended will be enrolled. Participants will complete questionnaires prior to initiating ET, then at 3, 6 and 12 months. The predictor variables are: 1) modifiable factors including illness and treatment cognitions, self-efficacy in coping with side effects anticipated or experienced, fear of breast cancer recurrence, trait anxiety, patient-physician interaction, 2) treatment-induced toxicities and 3) clinical factors including ET, chemotherapy, radiation, cancer stage and menopausal status. The outcome variable is adherence measured at each assessment post-treatment initiation with pill counts and participants’ self-report.

Inclusion criteria: 1) Histologically proven Stage I, II, IIIa EBC; 2) Treatment with ET (tamoxifen or an aromatase inhibitor).

Specific Aims: To evaluate the impact on adherence to ET by 1) potentially modifiable psychosocial factors 2) side effects and 3) clinical factors.

Statistical Method: Adherence responses will be transformed into dummy coded variables (0=take ET as prescribed; 1=does not take ET as prescribed). For each assessment, several separate univariate logistic regressions will be conducted with demographic (age, education, work and marital status, ethnicity), cognitive (illness and treatment perception), emotional (fear of recurrence and anxiety), coping (self-efficacy), and patient-physician relationship (role preference, trust and satisfaction), severity of side effects and clinical factors, as independent predictors of adherence. Only predictors which are significant at the 0.25 level will be entered in subsequent hierarchical multiple regression models to identify the main determinants of adherence.

Accrual: Expected study accrual is 200 over 12-18 months. Accrual started in May of 2011 and 36 patients have been accrued to the study.
OT3-02-02
Yoga Compared to Exercise as a Therapeutic Intervention during (Neo)adjuvant Chemotherapy in Women with Stage I-III Breast Cancer.

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Background: Psychological and physical distress is high in breast cancer patients receiving neoadjuvant or adjuvant chemotherapy. The patients’ ability to cope with this distress has an impact on treatment variables, i.e. deliverable chemotherapy dose, tolerability of side effects, and finally treatment completion rate. Exercise intervention studies have shown physiological and psychological benefits when undertaken during cancer treatment. There is also evidence that mind/ body interventions such as yoga are useful to manage treatment-related symptoms and anxiety in breast cancer patients. Considering the specific theoretical background of mind/body interventions this ongoing trial aims to elucidate the different effects of yoga and conventional exercise on physical and psychological factors in breast cancer patients undergoing neoadjuvant or adjuvant chemotherapy.

Trial design: Longitudinal data collection within an open, prospective, randomized trial using standardized questionnaires about inner correspondence and peacefulness with practices (ICPH), health-related quality of life (EORTC QLC C-30), fatigue (Cancer Fatigue Scale, CFS-D), mindfulness (Freiburg Mindfulness Inventory, FMI), spiritual/religious attitudes and disease coping (SpREUK), and life satisfaction (Brief Multidimensional Life Satisfaction Scale, BMLSS). Patients with newly diagnosed stage I-III breast cancer undergoing neoadjuvant or adjuvant chemotherapy are randomly assigned to receive yoga or conventional exercise on a 1:1 ratio. The yoga intervention consists of a weekly 60-minute iyengar-Yoga group-session together with individual home-based, self-contained 20-minute sessions twice a week. The conventional exercise intervention consists of a weekly 60-minute physiotherapy exercise session together with individual home-based, self-contained 20-minute sessions twice a week. Data assessments via questionnaires are done at baseline, right after the 12-week intervention period and 2 months after the end of intervention. Statistical analysis includes analyses of variance with all collected parameters and analysis of correlation between ICPH and above parameters. For statistical power 1-β=0.8 and two-sided probability of error α=0.05 the target accrual is 120 patients. Patient accrual within two breast care units started in April 2011 with 12 patients being on study to date (2011, June 21). Planned period of accrual is 20 months.

OT3-02-03
Patient Empowerment by Group Medical Consultations in the Follow-Up of Breast Cancer Survivors and Surveillance of Women with a BRCA Mutation.


Background: During usual follow-up care for breast cancer patients only little time is left for psychosocial support, while most patients experience several complaints, which may increase levels of distress or effect the quality of life. BRCA mutation carriers have a 40-80% life-time risk of developing breast cancer. They have a complex choice to make between yearly breast cancer surveillance or prophylactic mastectomy. Both options show increased survival rates.

To fulfill the needs for psychosocial support and information we have introduced group medical consultations (GMCs). A GMC provides individual medical visits conducted within a group. This 90 minute group-visit with 8-12 patients gives patients the opportunity to spend more time with their clinician and a behavioral health professional and learn from other patients experiencing similar topics. However, it should be noted that group sessions may increase fear in some patients.

Trial design: This multicenter randomized controlled trial will assess the effect of GMCs compared to individual visits for patients in the follow-up after breast cancer and for women with a BRCA mutation. The intervention group will participate in a GMC once, while the control group will have usual care.

Eligibility criteria: Inclusion criteria for the follow-up of breast cancer patients are: histologically proven breast cancer; age ≥18 years; primary treatment completed maximally 5 years ago. For BRCA mutation carriers inclusion criteria are: carrier of a BRCA1 or BRCA2 mutation; diagnosed maximally 2 years ago; age ≥25 years. Exclusion criteria for both groups are: currently involved in a diagnostic work-up because of a suspicion of breast cancer; metastatic breast cancer; current psychiatric disorder; insufficient command of the Dutch language. A history of prophylactic mastectomy is an exclusion criteria only for BRCA mutation carriers.

Study endpoints: Primary outcomes in this study are empowerment and psychological distress. In addition, the effects on cancer worry, information needs and information giving, self-examination of the breasts, treatment compliance, patient satisfaction, quality of life and costs of care (secondary outcome measures) will be studied.

Statistical methods: To assess a clinically relevant effect of GMCs on psychological distress with a power of 80%, a two-side significance of 5%, and a standard deviation of 45, 80 patients need to be included in each group. Randomization will take place per patient in blocks of 16 patients. Differences between baseline and post-test between the two groups will be compared by using ANCOVA for primary outcomes and ANOVA or mixed model for secondary analysis.

Accrual: Patients are currently being recruited at the Radboud University Nijmegen Medical Center. In the future other centers in the Netherlands will participate. Presently (21st June 2011) a total number of 37 patients have been included since start of the study (April 2011). The expected end of accrual of 320 patients will be December 2012.

OT3-02-04
TBCRC 012: ABCDE, a Phase II Randomized Study of Adjuvant Bevacizumab, Metronomic Chemotherapy (CM), Diet and Exercise after Preoperative Chemotherapy for Breast Cancer.

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Background: During usual follow-up care for breast cancer patients only little time is left for psychosocial support, while most patients experience several complaints, which may increase levels of distress or effect the quality of life. BRCA mutation carriers have a 40-80% life-time risk of developing breast cancer. They have a complex choice to make between yearly breast cancer surveillance or prophylactic mastectomy. Both options show increased survival rates.

To fulfill the needs for psychosocial support and information we have introduced group medical consultations (GMCs). A GMC provides individual medical visits conducted within a group. This 90 minute group-visit with 8-12 patients gives patients the opportunity to spend more time with their clinician and a behavioral health professional and learn from other patients experiencing similar topics. However, it should be noted that group sessions may increase fear in some patients.

Trial design: This multicenter randomized controlled trial will assess the effect of GMCs compared to individual visits for patients in the follow-up after breast cancer and for women with a BRCA mutation. The intervention group will participate in a GMC once, while the control group will have usual care.

Eligibility criteria: Inclusion criteria for the follow-up of breast cancer patients are: histologically proven breast cancer; age ≥18 years; primary treatment completed maximally 5 years ago. For BRCA mutation carriers inclusion criteria are: carrier of a BRCA1 or BRCA2 mutation; diagnosed maximally 2 years ago; age ≥25 years. Exclusion criteria for both groups are: currently involved in a diagnostic work-up because of a suspicion of breast cancer; metastatic breast cancer; current psychiatric disorder; insufficient command of the Dutch language. A history of prophylactic mastectomy is an exclusion criteria only for BRCA mutation carriers.

Study endpoints: Primary outcomes in this study are empowerment and psychological distress. In addition, the effects on cancer worry, information needs and information giving, self-examination of the breasts, treatment compliance, patient satisfaction, quality of life and costs of care (secondary outcome measures) will be studied.

Statistical methods: To assess a clinically relevant effect of GMCs on psychological distress with a power of 80%, a two-side significance of 5%, and a standard deviation of 45, 80 patients need to be included in each group. Randomization will take place per patient in blocks of 16 patients. Differences between baseline and post-test between the two groups will be compared by using ANCOVA for primary outcomes and ANOVA or mixed model for secondary analysis.

Accrual: Patients are currently being recruited at the Radboud University Nijmegen Medical Center. In the future other centers in the Netherlands will participate. Presently (21st June 2011) a total number of 37 patients have been included since start of the study (April 2011). The expected end of accrual of 320 patients will be December 2012.
exploring the efficacy of adjuvant combination chemotherapy and B. DFCI 05-055 (Mayer et al, ASCO 2007, 2008) demonstrated the feasibility of 1 year B after preoperative chemotherapy. Also, increasing data support risk reduction through lifestyle interventions (Segal, Ligibel et al, ASCO 2011). The ABCDE trial was designed to evaluate extended adjuvant B in a high risk post-preoperative cohort, and also assess the contribution of exercise to a dietary intervention.

**Eligibility Criteria**
Eligible pts have HER2- breast cancer and have received preoperative anthracycline and/or taxane-based chemotherapy with residual invasive disease at surgery. Acceptable stages include: triple negative if preop stages I-III, or ER+/PR+ if stage III preop or IIIB postop. Acceptable organ function and standard B exclusions apply. Registration must occur between 28-180 days after last surgery.

**Specific Aims**
Primary endpoint is recurrence-free survival at a median follow-up of 6 years. Secondary endpoints include B pharmacogenomics, evaluation of the impact of exercise on quality of life and biomarkers associated with recurrence, and prospective examination of cardiac toxicity. Residual tissue-based predictors of outcome will be extensively explored, including PAM50, Ki67, and VEGF hypoxia signature.

**Methods**
This is a 2 x 2 randomized study with a first randomization to 6 months (mo) B 15 mg/kg every 3 weeks (wks) plus 6 mo CM (C 50 mg daily, M 2.5 mg twice daily days 1, 2 each wk), followed by 2.5 years B 15 mg/kg every 6-8 wks, versus observation. A second randomization is to a 1 year telephone-based lifestyle intervention, offering dietary modification alone, or in combination with a structured exercise program.

**Statistical Methods and Accrual**
Total sample size is 660 pts within the Translational Breast Cancer Research Consortium. Overall power is 0.80 to detect a hazard ratio of 0.59-0.68, depending on pt population. Accrual initiated early 2011 and is expected to continue for the next 36 months.

**Conclusions**
Patients with residual disease after preoperative chemotherapy are at high risk of recurrence and have unmet medical needs. To our knowledge, this is the only trial testing a prolonged but less intensive adjuvant B schedule in this clinical setting. Results of this study could have critical implications for the management of this patient population and for the design of future clinical trials with anti-angiogenic agents.
Najita, J P2-18-02
Nguyen, B P5-11-09
Nicholson, RJ P1-01-19
Nakagawa, F P1-15-01
Nakagawa, K P5-06-05
Nakahara, S P5-07-46
Nakagawa, Y P5-07-16
Nakamura, S P4-16-02, P4-20-05
Nakamura, Y P5-13-21
Nakatsuka, M P1-11-16
Nakatsuka, M P1-11-16
Nakatsuji, T P1-01-10, P1-01-11
Nakayama, S P2-13-02, P2-12-27
Namas, B P4-11-07
Narayanan, M P1-06-23
Narka, S P2-10-10
Narayanan, A P4-06-06
Nasir, N P5-05-06
Nascimento, YV P3-07-37
Nasem, M P5-08-04
Nashed, M P1-11-11
Nash, M P1-11-11
Nasr, M P4-01-04
Nasr, N P1-06-12
Nastala, C P2-16-12
Nasu, H P2-14-03, P3-07-44, P3-14-03, P3-14-16,
P3-10-04
Nattrajan, A P4-20-05
Nattrajan, R P1-06-22
Natter, C P4-02-02
Nama, B P4-06-05, P4-06-06
Nawrocki, E P4-17-05
Nay, B P3-14-01
Nazar, A P5-07-16, P5-13-06
Nazarí, A P5-05-07
Nazarío, ACP P4-02-10, P5-07-06, P5-13-06
Nearchou, A P3-07-34
Necchi, RM P5-09-03
Nechutia, H P5-09-03
Nechuta, SJ P1-08-02
Necoziens, S P4-08-02
Nedermeyer, M P2-14-19
Nederer, PM P3-03-08
Nell, T P2-06-04
Negrón, V P4-02-03
Nekljudova, V S2-4, S3-6, S4-8
Nel, M P5-15-05
Nelson, JD P4-06-05
Nelson, JT P4-06-06, P5-18-07
Nelson, MA P6-14-06, MS1-2
Nelson, M P1-11-08
Nebauer, MA P5-06-05, P5-19-09
Nebauer, S P3-13-06
Neben, J P5-09-03
Nebewug, A S3-1, P4-02-05
Nebrün, H S6-3, P6-04-04, P4-12-03
Neel, D P5-06-05
Neeb, B P5-16-08
Newall, D P4-03-05
Newbold, RF P4-01-17
Newell, J P1-09-05, P2-12-15
Newell, JT P1-09-05, P2-12-15
Newitt, DC P2-09-07
Newman, V P4-08-05
Newman, WD P4-11-07
Newman, W P4-11-07
Newman, W P4-11-07
Newhart, JA P3-07-27, P3-12-03, P5-13-21,
P3-17-03, P5-06-09
Newman, SC P1-06-03
Newnham, E P5-15-05
Newnham, M P5-13-06
Newnham, VM P5-13-06
Ney, A P5-13-06
Ney, F P4-01-17
Ney, H P1-07-30
Ney, J P1-06-09
Ney, S P1-06-05
Ney, T P5-14-01
Ney, T P5-14-01
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