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23nd Annual San Antonio ***Breast Cancer Symposium***

DECEMBER 6-9, 2000

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CONFERENCE GRANTS

National Cancer Institute (1R13 CA83702-02)
American Cancer Society, Texas Division

Symposium Dates

2001:	December 10-13
2002:	December 11-14
2003:	December 3-6
2004:	December 8-11

Abstract Submission Deadline: June 1 every year

WEDNESDAY, DECEMBER 6

9:00-9:15 WELCOME

Opening Remarks
Charles A. Coltman, Jr., MD

9:15-12:00 MINI-SYMPOSIUM I

Microarray-Based Analysis of Breast Cancer
Peter O'Connell, PhD, Moderator
Baylor College of Medicine, Houston, Texas

9:15 **Introduction**

9:15 **Array-Based Comparative Genomic Hybridization Analysis of Breast Cancer**
Joe W. Gray, PhD
University of California
San Francisco, California

9:50 **In Vivo Gene Expression Profiling of Human Breast Cancer Progression**
Abdel G. Elkahloun, PhD
National Human Genome Research Institute, NIH
Bethesda, Maryland

10:25 **Molecular Characterization and Classification of Human Breast Tumors Using cDNA Microarrays**
Charles M. Perou, PhD
Stanford University
Stanford, California

11:00 **Translation of Array Results to the Clinic**
Susan G. Hilsenbeck, PhD
Baylor College of Medicine
Houston, Texas

11:35 **Panel Discussion**

12:00-1:00 LUNCH [Ticket Required]

1:00-2:00 WILLIAM L. MCGUIRE MEMORIAL LECTURE

Molecular Markers in Breast Cancer: Where Are We, Where Are We Going?
Trevor J. Powles, MD PhD
Royal Marsden Hospital
Sutton, Surrey, England, UK

Supported by an educational grant from Glaxo Wellcome

2:00-5:00 GENERAL SESSION I

2:00 **1. Early Results of Breast Cancer Lymphatic Mapping from the H. Lee Moffitt Cancer Center: No Axillary Recurrences in Breast Cancer Patients after a Negative Sentinel Lymph Node Biopsy.**
Dessureault S, Dupont E, Shons A, Berman C, Ku NN, Cox C, Reintgen D. H. Lee Moffitt Cancer Center and Research Institute at the University of South Florida, Tampa, FL.

2:15 **2. Axillary Node Examination Is a Weaker Indicator of Metastatic Potential for Inner Quadrant Compared to Outer Quadrant Breast Cancers.**
Tan WW, Herman TS, Ravdin PM. University of Texas Health Science Center, San Antonio, TX.

2:30 **3. Identification of Occult Metastatic Cells in Bone Marrow Predicts Poor Prognosis Better Than HER2/neu Status and Angiogenesis in Breast Carcinomas.**
Braun S, Schindlbeck C, Schaffer P, Atkinson R. Technical Univ., Munich, Germany; Ludwig-Maximilians Univ., Munich, Germany; Univ. of Southern California, Los Angeles, CA.

2:45 **4. Selection of High Risk Lymph Node Negative Breast Cancer Patients for Chemotherapy Can Best Be Done with the Mitotic Activity Index.**
Van Diest PJ, Baak JPA, and other MMCP Collaborators of Pathology. Free University Hospital, Amsterdam, The Netherlands.

3:00 **5. Indicators of Lifetime Estrogen Exposure: Effect on Breast Cancer Incidence and Interaction with Raloxifene Therapy in MORE Trial Participants.**
Lippman ME, Krueger KA, Eckert S, Cauley JA, Walls EL, Jamal S, Cummings SR, and the Prevention Sciences Group. Lombardi Cancer Center, Georgetown University Medical Center; Eli Lilly and Co.; University of Pittsburgh; Women's College Hospital, Toronto; UCSF.

3:15 **6. Comparison of Efficacy and Tolerability of Fulvestrant (Faslodex™) with Anastrozole (Arimidex™) in Post-Menopausal Women with Advanced Breast Cancer – Preliminary Results.**
Howell A, Robertson JFR, Quaresma Albano J, Aschermannova A, Mauriac L, Kleeberg UR, Vergote I, Erikstein B, Webster A, Morris C. Christie Hospital, Manchester, United Kingdom; City Hospital, Nottingham, United Kingdom; Instituto De Oncologicia, Coimbra, Portugal; Odborny Lecebny Ustav Onkol., Nova Ves Pod Plesi, Czech Republic; Institut Bergonnie, Bordeaux, France; Haemat./Onkol. Praxis, Hamburg, Germany; University Hospital, Leuven, Belgium; Radiumhospital Onkologisk Aveling, Oslo, Norway; AstraZeneca, Macclesfield, United Kingdom.

3:30 **7. A Double-Blind Randomized Trial Comparing the Efficacy and Tolerability of Faslodex™ (Fulvestrant) with Arimidex™ (Anastrozole) in Post-Menopausal Women with Advanced Breast Cancer.**
Osborne CK, on Behalf of the North American Faslodex Investigator Group. Baylor College of Medicine, Houston, TX.

3:45 **8. Femara® (Letrozole) Showed Significant Improvement in Efficacy over Tamoxifen as First-Line Treatment in Postmenopausal Women with Advanced Breast Cancer.**
Smith R, Sun Y, Garin A, Fein L, Sleeboom HP, Chaudri H, Dugan M, Staffler B, Brady C, on Behalf of the Letrozole International Breast Cancer Study Group. South Carolina Oncology Associates, Columbia, SC; Cancer Hospital, Beijing, China; Cancer Research Center, Moscow, Russia; Centro Oncologico, Rosario, Argentina; Ziekenhuis Leyenburg, Den Haag, The Netherlands; Novartis Pharma, Basel, Switzerland.

4:00 **9. Sulfotransferase Expression in Normal and Cancerous Human Breast Tissues.**
Falany CN, Wang J, Falany JL, Frost AR. University of Alabama at Birmingham, Birmingham, AL.

4:15 **10. Resistance of Mutant BRCA1 Breast Cancer Cells to Paclitaxel-Induced Apoptosis Mediated by Bcl-2.**
Turner BC, Ren Q, Gupta PK, Basu A, Krajewski S, Krajewska M, Potoczek M, Carbone CJ, Reed JC, Haldar S. Thomas Jefferson University Hospital, Philadelphia, PA; Case Western Reserve University, Cleveland, OH; The Burnham Institute, La Jolla, CA.

4:30 **11. Akt2 Upregulation in HER-2/neu Overexpressing Breast Cancers: Implications to Their Clinical and Biological Behavior.**
Bacus SS, Esteva FJ, Hortobagyi G, Gudkov AV. Quantitative Diagnostics Laboratory, Elmhurst, IL; UT MD Anderson Cancer Center, Houston, TX; University of Illinois College of Medicine, Chicago, IL.

4:45 **12. Telomerase Activity in Human Breast Cancer.**
Mokbel K, Hu JCC, Kirkpatrick K, Ghilchik M, Parris C, Newbold R. St Bartholomew's Hospital, London, United Kingdom.

5:00-7:00 POSTER SESSION I & RECEPTION

Detection/Diagnosis

Axillary/Sentinel Nodes

101 **The Almanac Trial: Initial Experience.**
Mansel RE, on Behalf of ALMANAC Collaborators Group. Cardiff, United Kingdom.

102 **Physiologic and Socioeconomic Consequences of Breast Cancer Surgery: A Comparison between Sentinel Lymph Node Biopsy and Axillary Lymph Node Dissection.**
Burak WE Jr., Hollenbeck ST, Zervos EE, Young DC. The Ohio State University, Arthur G. James Cancer Hospital, and Richard J. Solove Research Institute, Columbus, OH.

103 **Quantitative Real-Time RT-PCR Detection of Breast Cancer Micrometastasis Using a Multi-Gene Marker Panel.**
Mitas M, Mikhitarian K, Walters C, Baron P, Elliot B, Brothers T, Robison J, Metcalf JS, Gillanders WE, Cole DJ. Medical University of South Carolina, Charleston, SC.

104 **Detection of Axillary Node Positivity Is Increased with Sentinel Lymph Node Biopsy in Early Stage Breast Carcinoma.**
Bevilacqua JLB, Van Zee KJ, Mann GB, Tan LK, Cody HS III, Heerdt AS, Montgomery LL, Petrek J, Borgen PI, Port ER. Memorial Sloan-Kettering Cancer Center, New York, NY.

105 **The First 2,000 Cases - Axillary Node Metastases in the Sentinel Lymph Node Era.**
Bevilacqua JLB, Tan LK, Borgen PI, Cody HS III, Heerdt AS, Montgomery LL, Petrek JA, Van Zee KJ. Memorial Sloan-Kettering Cancer Center, New York, NY.

106 **RT-PCR Analysis for Mammaglobin and Carcinoembryonic Antigen Detects Metastases in Histology-Negative Sentinel Lymph Nodes.**
Verbanac KM, Min CJ, Purser SM, Swanson MS, Lo K, Albrecht JA, Tafra L. East Carolina University, Greenville, NC; National Genetics Institute, Los Angeles, CA; Anne Arundel Medical Center, Annapolis, MD.

107 **Variations of Success Rates for Sentinel Node Mapping in Breast Cancer: Results of a German Multicenter Study.**
Kuehn T, Kotzerke J, Santjohanser C, Schirrmeister H, Grimm S, Koretz K, Rebstock AB, Reske SN, Kreienberg R. University Medical Center of Ulm, Germany.

108 **A New Score for the Assessment of Postsurgery Morbidity Following Axillary Surgery.**
Kuehn T, Klauss W, Darsow M, Regele S, Flock F, Wendt I, Rebstock AB, Kreienberg R. University Medical Center of Ulm, Germany.

109 **Long Term Results of Randomised Studies of Axillary Clearance Vs Non-Targeted Axillary Sampling.**
Lambah PA, Dixon JM, Prescott RJ, Jack W, Forrest APM, Chetty U, on Behalf of the Edinburgh Breast Unit. Western General Hospital, Edinburgh, Scotland, United Kingdom.

110 **The Clinical Significance of Microinvasive Breast Cancer.**
Maibenco DC, Weiss LK, White JJ, Schwartz AG, Kau TY, Severson RK. Surgical Specialists of Decatur, Decatur, IL; Karmanos Cancer Institute, Detroit, MI.

111 **Intraoperative Assessment of the Sentinel Node in Breast Cancer: Comparison of Frozen Sections Using Immunohistochemistry and H+E Sections with Traditional Postoperative Assessment.**
Khonji NI, Clarke D, Goyal S, Douglas-Jones AG, Jasani B, Sweetland HM, Mansel RE. University of Wales College of Medicine, Cardiff, United Kingdom; University Hospital of Wales, Cardiff, United Kingdom.

112 **Effect of Blue Dye on Pulse Oximetry during Sentinel Node Biopsy.**
Khonji NI, Clarke D, Goyal S, Mukhtar Z, Al-Rawi K, Sweetland HM, Mansel RE. University of Wales College of Medicine, Cardiff, United Kingdom.

113 **The Intradermal Sentinel Node: Update 2000.**
Owen DH. Betty Seinfeld Breast Ctr., Boyton Beach, FL.

114 **Predictive Value of the Sentinel Lymph Node in Breast Cancer: How Many Sentinel Nodes Are Enough?**
Zervos EE, Farrar WB, Walker MJ, Yee LD, Young DC, Burak WE. Arthur G. James Cancer Center at The Ohio State University, Columbus, OH.

115 **Effect of Technetium 99m Sulphur Colloid Injection Interval (TII) on Sentinel Node Biopsy.**
Chua AN, Lannin DL, Swanson MS, Tafra L. East Carolina University, Greenville, NC; Anne Arundel Medical Center, Annapolis, MD.

116 **Predictors of Nonsentinel Node Metastasis in Breast Cancer Patients: Size and Extracapsular Extension of the Sentinel Node Metastasis.**
Stitzenberg KB, Calvo BF, Neelon BH, Iacocca MV, Ollila DW. University of North Carolina, Chapel Hill, NC.

117 **Patterns of Internal Mammary Drainage during Sentinel Node Lymphoscintigraphy.**
Smitt MC, Bevan A, Strauss HW, Jeffrey SS. Stanford University, Stanford, CA.

Prognosis and Response Prediction

Prognostic Factors

118 **Prognostic Effects of Circulating Insulin-Like Growth Factor Binding Proteins (IGFBP's) 1 and 3 in Operable Breast Cancer.**
Goodwin PJ, Ennis M, Trudeau ME, Koo J, Pritchard KI, Hartwick W, Hood N, Hoffman B. Samuel Lunenfeld Research Institute at Mount Sinai Hospital; Sunnybrook and Women's College Health Science Center; St. Michael's Hospital; University of Toronto, Canada.

119 **Urokinase Plasminogen Activator (uPA) and Its Type-1 Inhibitor (PAI-1) Are Superior to the Nottingham Prognostic Index NPI in Predicting Relapse in Node-Negative Breast Cancer Patients.**
Lisboa BW, Friedrichs K, Riethdorf L, Löning T, Jänicke F. University Hospital, Hamburg, Germany.

120 **Diabetes but Not Obesity Is a Prognostic Factor for Disease Free Survival in Women with Stage I, II, or III Breast Carcinoma Receiving Tamoxifen.**
Song EY, Banerjee M, Du W, Hryniuk WM. Wayne State University, Detroit, MI; Karmanos Cancer Institute, Detroit, MI.

121 **Elevated Cytosol Vascular Endothelial Growth Factor (VEGF) Showed a Significant Influence on Outcome: Retrospective Analysis of 173 Patients with Stage I and II Breast Cancer.**
Bauerfeind IG, Hagen D, Konecny G, Kahlert S, Boettcher B, Nestle-Kraemling C, Untch M. Ludwig-Maximilians-University Munich, Klinikum Großhadern, Munich, Germany.

122 **Age-Dependent Breast Cancer Prognostic Markers.**
Eppenberger-Castori S, Moore D, Quong J, Thor A, Eppenberger U, Benz CC. University of California, San Francisco, CA; Northwestern University, Evanston, IL; Stiftung Tumorbank Basel, Basel, Switzerland.

123 **Expression of Estrogen Receptor β Protein in Human Breast Cancer: Correlation with Clinical Tumor Parameters.**
Fuqua SAW, Schiff R, Parra I, Moore JT, Mohsin S, Clark GM, Allred DC. Baylor College of Medicine, Houston, TX; Glaxo Wellcome Research and Development, Research Triangle Park, NC.

124 **c-erbB-2 as a Prognostic and Predictive Factor in Breast Cancer: A Meta-Analysis.**
Trock BJ, Yamauchi H, Brotzman M, Stearns V, Hayes DF. Georgetown University, Washington, DC.

125 **Clinical Impact of Two New Prognostic Factors (VEGF and $p27^{kip1}$) on Conventional Risk Categories for Node-Negative Breast Cancer.**
Coradini D, Pellizzaro C, Benini E, Daidone MG. Istituto Nazionale Tumori, Milano, Italy.

126 **The Subcellular Localisation of Cyclin B, Cdc2 and $p21^{WAF1/CIP1}$ in Breast Cancer: Association with p53 Status and Prognosis.**
Winters ZE, Hunt NC, Bradburn M, Royds J, Harris AL, Norbury CJ. University of Bristol, Bristol, United Kingdom; John Radcliffe Hospital, Oxford, United Kingdom; University of Sheffield Medical School, Sheffield, United Kingdom; University of Oxford, Institute of Molecular Medicine, Oxford, United Kingdom.

127 **Validation of the Prognostic Significance of Mitosin in Node-Negative Patients.**
Harvey JM, Allred DC, Chamness GC, Osborne CK, Clark GM. University of Western Australia, Nedlands, Western Australia, Australia; Baylor College of Medicine, Houston, TX.

128 **The Complex between Urokinase and Its Type-1 Inhibitor in Primary Breast Cancer: Relation to Survival.**
Pedersen AN, Christensen IJ, Stephens RW, Briand P, Mouridsen HT, Danø K, Brünner N, and the Danish Breast Cancer Cooperative Group. Finsen Laboratory, Copenhagen, Denmark; Danish Cancer Society, Copenhagen, Denmark.

129 **Tissue Arrays for the Molecular Profiling of Inflammatory Breast Cancers.**
Chang J, Clark GM, Mohsin S, Allred DC, Elledge RM. Baylor College of Medicine, Houston, TX.

130 **Prognostic Significance of a Novel Hypoxia Regulated Marker, Carbonic Anhydrase IX (MN/CA IX), in Invasive Breast Cancer.**
Chia SK, Watson PH, Wykoff CC, Leek RD, Han C, Pastorek J, Gatter KC, Ratcliffe P, Harris AL. British Columbia Cancer Agency, Vancouver, BC, Canada; University of Manitoba, Winnipeg, MB, Canada; University of Oxford, Oxford, United Kingdom; Institute of Virology, Slovakia.

131 **Data from the SEER (Surveillance, Epidemiology, and End Results) DataBase Demonstrates the Validity of Using Routine Histologic Grading for Prognostic Assessment of Patients with Early Breast Cancer.**
Khan Q, Ravdin PM. University of Texas Health Science Center, San Antonio, TX.

132 **Breast Cancer in Young Women Is Associated with a Worse Prognosis: A Case-Control Study.**
Kalfon B, Gu Y, Fineberg S, Sparano JA. Albert Einstein Comphrensive Cancer Center, Bronx, NY.

133 **Effect of Elevated Serum Carboxyterminal Telopeptide (ICTP) on Survival in Breast Cancer Patients with Only Bone Metastases.**
Ali SM, Leitzel K, Demers L, Chinchilli V, Engle L, Costa L, Lipton A. Penn State Univ., Hershey, PA; VA Medical Center, Lebanon, PA; Hospital Santa Maria, Lisbon, Portugal.

134 **The Impact of Young Age on Short-Term and Long-Term Clinical Course of Local Relapse in Early Breast Cancer.**
Broët P, De Rycke Y, Moreau T, Asselain B. Institut Curie, Paris, France; INSERM, Villejuif, France.

135 **Overexpression of Bcl-2 and Bax in Breast Cancer: Correlation to Established Prognostic Factors.**
Langer-Nitsche C, Lück HJ, Schroers U, Linge G, Kühnle H. University Hospital and Medical School, Hannover, Germany.

136 **Factors Influencing the Effect of Age on Prognosis in Breast Cancer: A Population Based Study.**
Sainsbury R, Haward B. Royal Infirmary, Huddersfield; University of Leeds, Leeds, United Kingdom.

137 **High Levels of Cathepsin D and c-erbB-2 Oncoprotein in Low Histological Grade Tumors Defines a Subgroup of Node-Negative Breast Cancer Patients with High Risk of Relapse.**
Gaci Z, Ingrand P, Gaci M, Bouin-Pineau MH, Daban A, Metaye T. University Hospital, Poitiers, France.

138 **Morphological Characteristics of Metastatic Nodes Are Important as a New Prognostic Indicator.**
Sakamoto G, Akiyama F, Kasumi F, Hatakeyama K. Niigata University, Niigata, Japan; Cancer Institute, Tokyo, Toshima-ku, Tokyo, Japan; Cancer Institute, Tokyo, Toshima-ku, Tokyo, Japan.

139 **Locally Advanced Breast Cancer: Prognostic Variables Affecting Results.**
Baldini E, Gardin G, Lionetto R, Montanaro E, Prochilo T, Rosso R, Conte PF. Dipartimento di Oncologia U.O. di Oncologia Medica, Pisa; Istituto Scientifico Tumori (IST), Genova, Italy.

140 **Nuclear Grade and Tumor Marker Expression in Tubular Breast Carcinoma.**
Gupta R, Shah RN, Wiley EL, Badve S. Northwestern University, Chicago, IL.

141 **Biological Factors in Primary Breast Cancer of Young Women (≤ 35 Years).**
van de Pol S, Thunnissen E, Joosten-Achjanie S, Wagstaff J, Hupperets P. University Hospital Maastricht, Maastricht, AZ, The Netherlands.

142 **Amplification of HER-2 neu Predicts for Poorer Survival in Locally Advanced Breast Cancer.**
Cameron DA, Bartlet JMS, Watters AD, Leonard RCF. Western General Hospital, Edinburgh, Scotland, United Kingdom; Glasgow Royal Infirmary, Glasgow, Scotland, United Kingdom.

143 **Traditional and New Prognostic Factors in Locally Advanced Breast Cancer.**
Behrens K, Thomssen C, Kahlert S, Sattler D, Kuhn W, Oberlechner E, Lebeau A, Dettmer P, Konecny G, Jaenicke F, Untch M. University Hospital, Hamburg; University Hospital Grosshadern, Munich; Technical University, Munich; Hospital Landshut, Germany.

144 **18F-FDG-Positron Emission Tomography and Breast Cancer: Does FDG-Uptake Correlate with Prognostic Markers?**
Buck AK, Kühn T, Schirrmeister H, Glatting G, Reske SN. University of Ulm, Ulm, Germany.

145 **Micrometastases in Axillary Lymph Nodes Detected by RT-PCR as a Valuable Prognostic Factor in Node-Negative Breast Cancer Patients.**
Norikazu M, Yasuhiro T, Isao S, Masaru O, Tadashi O, Takashi M, Masao K, Noriko A, Kousaku O, Morito M. Graduate School of Medicine, Osaka University, Suita, Osaka, Japan; Osaka University, Suita, Osaka, Japan.

Risk and Prevention

Prevention

146 **Efficacy of Prophylactic Mastectomy in Unaffected BRCA1/2 Mutation Carriers: First Prospective Study.**
Klijn JG, Verhoog LC, Brekelmans C, van Geel AN, Menke M, Seynaeve C, Tilanus-Linthorst M, Bartels C, van den Ouweland A, Burger C, Meijers-Heijboer EJ. Dr. Daniel den Hoed Cancer Center and University Hospital Rotterdam, Erasmus University Rotterdam, The Netherlands.

147 **Study of the Biological Effects of the Aromatase Inhibitor Letrozole in Healthy Postmenopausal Women: Rationale for Prevention.**
Harper-Wynne CL, Ross GM, Sacks NP, Gui GP, Dowsett M. The Royal Marsden Hospital, Fulham, London, United Kingdom.

148 **Chemopreventive and Chemotherapeutic Effects of Tamoxifen and 9 Cis Retinoic Acid in the MNU-Induced Rat Mammary Tumor Model.**
Lubet RA, Christov K, Steele VE, Kelloff GJ, Hill DJ, Grubbs CJ. NCI/DCP Bethesda, MD; Univ. Ill., Chicago; Univ of Alabama, Birmingham, AL.

149 **Phase II Chemoprevention Trial of DFMO Using the Random FNA Model.**
Fabian CJ, Kimler BF, Brady D, Zalles CM, Mayo MS, Masood S, Grizzle WE. University of Kansas Medical Center, Kansas City, KS; University of Florida, Jacksonville, FL; University of Alabama Cancer Center, Birmingham, AL.

150 **Safety of a Gonadotropin-Releasing Hormone Agonist (GnRHA)-Based Hormonal Chemoprevention Regimen for Young Women at High Genetic Risk for Breast Cancer.**
Weitzel JN, Pike MC, Daniels AM, Ursin G, Daniels JR, Spicer DV. City of Hope National Medical Center, Duarte, CA; University of Southern California School of Medicine, Los Angeles, CA; Balance Pharmaceuticals, Inc., Santa Monica, CA.

151 **Identifying Subjects for Breast Cancer Risk Reduction: An Epidemiologic Approach.**
Vogel VG, Costantino JP. Univ. of Pittsburgh Cancer Institute, Pittsburgh, PA.

152 **A Randomized Trial of Fenretinide in HRT Users Using IGF-I as a Surrogate Biomarker.**
Bonanni B, Ramazzotto F, Franchi D, Buttarelli M, Valente I, Stegher C, Daldoss C, Pigatto F, Mora S, Cazzaniga M, Pizzamiglio M, Pelosi G, Decensi A. European Institute of Oncology, Milan, Italy; University of Brescia, Italy; University of Varese, Italy; Centro Neoplasie Femminili and Buzzi Hospital, Milan, Italy.

Diet and Nutrition

153 **Effects of Dietary Flaxseed in Women with Cyclical Mastalgia.**
Goss PE, Li T, Theriault M, Pinto S, Thompson L. University Health Network/Princess Margaret Hospital, Toronto, ON, Canada; University of Toronto, Toronto, ON, Canada.

154 **The Women's Healthy Eating and Living (WHEL) Study: A Nutritional Intervention Study in Breast Cancer Survivors.**
Jones VE, Hollenbach K, Rock C, Faerber S, Haan M, Gold E, Thomson C, Marshall J, Stafnick M, Caan B, Jones L, Hajek R, Ritenbaugh C, Pierce J. University of California San Diego, San Diego, CA; University of California Davis, Davis, CA; University of Arizona, Tucson, AZ; Northern California Cancer Center, Union City, CA; Kaiser Permanente, Oakland, CA; University of Texas M.D. Anderson Cancer Center, Houston, TX; Center for Health Research, Portland, OR.

155 **Comparison of Dietary Assessment Methods in a Low Fat Dietary Intervention Program for Women at High Risk for Breast Cancer.**
Simon MS, Uhley V, Djuric Z, Lababidi S, Depper J, Kresge C, Klurfeld DM, Heilbrun L. Barbara Ann Karmanos Cancer Institute, Detroit, MI.

156 **Weight Loss in Obese Breast Cancer Survivors: Novel Strategies.**
Djuric Z, DiLaura N, Jenkins I, Mood D, Jen C, Hryniuk W. Karmanos Cancer Institute, Detroit, MI.

157 **Biological Effects of Dietary Flaxseed in Patients with Breast Cancer.**
Thompson LU, Li T, Chen J, Goss PE. University of Toronto, Toronto, ON, Canada; Princess Margaret Hospital, Toronto, ON, Canada.

158 **Evidence for TGFβ-Mediated Growth Inhibition by Genistein in Human Mammary Epithelial Cells.**
Xu J, Peterson G, Su Y, Murphy-Ullrich J, Kim H, Barnes S. University of Alabama at Birmingham, Birmingham, AL.

159 **A Randomized, Placebo-Controlled, Double-Blinded Clinical Trial of a Soy Beverage in the Treatment of Hot Flushes in Breast Cancer Survivors.**
Kutynec CL, Olivotto IA, Prior JC, Hislop TG, Chambers KG, Gelmon KA, Templeton E. British Columbia Cancer Agency, University of British Columbia, Vancouver General Hospital, Vancouver, BC, Canada.

Treatment

Endocrine Therapy

160 **Recombinant Human Chorionic Gonadotropin (r-hCG) Reduces the Incidence and Progression of Mammary Tumors in the Rat.**
Russo IH, Slater C, Lareef MH, Mihaila D, Ao X, Quillen DP, Arulanandam ARN, Russo J. Fox Chase Cancer Center, Philadelphia, PA; Serono, Randolph, MA.

161 **Recombinant Human Chorionic Gonadotropin (r-hCG) Significantly Reduces Primary Tumor Cell Proliferation in Patients with Breast Cancer.**
Russo J, Janssens JPh, Russo IH. Fox Chase Cancer Center, Philadelphia, PA; Willems Inst.

162 **Efficacy of Tamoxifen Following Arimidex™ (Anastrozole) as First-Line Treatment for Advanced Breast Cancer in Postmenopausal Women.**
Thurlimann B, Robertson JFR, Bonneterre J, Buzdar A, Nabholtz J-MA, on Behalf of the Arimidex Study Group. Medizinische Klinik C Kantonsspital, for the Swiss Group for Clinical Cancer Research SAKK (President: A Goldhirsch), St Gallen, Switzerland; City Hospital, Nottingham, United Kingdom; Centre Oscar Lambret, Lille, France; MD Anderson Cancer Center, Houston, TX; Cross Cancer Institute, Edmonton, AB, Canada.

163 **SCH 57058 Is a Selective Estrogen Receptor Modulator (SERM) without Uterotrophic Effects Compared with Either Tamoxifen or Raloxifene.**
Johnston SRD, Detre S, Riddler S, Dowsett M. Royal Marsden Hospital, London, United Kingdom; Institute of Cancer Research, Sutton, United Kingdom.

164 **The Effect of Anastrozole (Arimidex™) on Serum Lipids – Data from a Randomized Comparison of Anastrozole Vs Tamoxifen in Postmenopausal Women with Advanced Breast Cancer.**
Dewar J, Nabholtz J-MA, Bonneterre J, Buzdar A, Robertson JFR, Thurlimann B, Clack G. Ninewells Hospital, Dundee, United Kingdom; Cross Cancer Institute, Edmonton, AB, Canada; Centre Oscar Lambret, Lille, France; MD Anderson Cancer Center, Houston, TX; City Hospital, Nottingham, United Kingdom; Medizinische Klinik C Kantonsspital, St Gallen, Switzerland; AstraZeneca Pharmaceuticals, Alderley Park, United Kingdom.

165 **Clinical and Endocrine Data for Goserelin (Zoladex) Plus Anastrozole (Arimidex) as Second Line Endocrine Therapy in Premenopausal Women with Advanced Breast Cancer.**
Forward DP, Cheung KL, Jackson L, Robertson JFR. Nottingham City Hospital, Nottingham, United Kingdom.

166 **Pre-Clinical Pharmacology Profile of a New Selective Estrogen Receptor Modulator (SERM), ERA-923, for the Treatment of ER Positive Breast Cancer.**
Greenberger LM, Komm B, Miller C, Annable T, Lyttle R, Frost P, Satyaswaroop PG. Wyeth-Ayerst Research, Pearl River, NY; Wyeth-Ayerst Research, Radnor, PA; Pennsylvania State University, Hershey, PA.

167 **Promising Activity and Safety of Exemestane as First-Line Hormonal Therapy in Metastatic Breast Cancer Patients: Final Results of an EORTC Randomised Phase II Trial.**
Paridaens R, Dirix L, Lohrisch C, Beex L, Nooij M, Cameron D, Biganzoli L, Cufer T, Yague C, Lobelle JP, Piccart M. EORTC, Brussels, Belgium; Pharmacia & Upjohn, Brussels, Belgium.

168 **Endometrial Histopathology in 700 Patients Treated with Tamoxifen for Breast Cancer.**
Penault-Llorca FM, Delatour M, Le Bouedec G, Kalir T, Cohen CJ, Dauplat J, Deligdisch L. Centre Jean Perrin, Clermont-Ferrand, France; Mount Sinai - NYU School of Medicine, New York, NY.

169 **Intra-Tumoral Variation in Pathological Assessments of Apoptosis and Proliferation in Breast Cancer Biopsies.**
Cameron DA, Marson L, Iqbal S, Dawson L, Anderson TJ, Dixon M, Miller BR. Western General Hospital, Edinburgh, Scotland, United Kingdom.

170 **Exemestane as Neoadjuvant Treatment for Locally Advanced Breast Cancer: Endocrinologic and Clinical Endpoints.**
Dixon JM, Grattage L, Renshaw L, Miller WR. Western General Hospital, Edinburgh, Scotland, United Kingdom.

171 **Letrozole Is Highly Effective in Patients with Soft Tissue Metastases.**
Possinger K, Schmid P, Wischnewsky MB. Charité Campus Mitte, Humboldt University Berlin, Berlin, Germany; University of Bremen, Bremen, Germany.

172 **A Comparison of the Single-Dose Pharmacokinetics of 'Faslodex' (Fulvestrant) 250 mg When Given as Either a One x 5-ml Intra-Muscular (i.m.) Injection or Two x 2.5-ml Injections in Postmenopausal Women with Advanced Breast Cancer.**
Robertson JFR. City Hospital, Nottingham, United Kingdom.

173 **Anastrozole Vs Tamoxifen in Hormonodependent Advanced Breast Cancer. A Phase II Randomized Trial.**
Milla-Santos A, Milla L, Rallo L, Solano V. Ntra. Sra. del Pilar Hosp., Barcelona, Spain.

174 **Letrozole and Megestrole Acetate in Patients with Advanced Breast Cancer Resistant to Tamoxifen.**
Wischnewsky MB, Schmid P, Boehm R, Verbeek JA, Possinger K. University, Bremen, Germany; Charite, Berlin, Germany; Novartis, Basel, Switzerland.

Tumor Cell Biology

Gene Therapy

175 **Adenovirus Mediated Expression of E2F-1 in Combination with Paclitaxel Has a Synergistic Effect on Breast Cancer Growth Inhibition.**
Yoshida K, Nishizaki M, Hunt KK. University of Texas M. D. Anderson Cancer Center, Houston, TX.

Oncogenes/Tumor Suppressor Genes

176 **Mechanisms of HET/SAF-B Mediated Repression of Estrogen Receptor's Transcriptional Activity.**
Townson SM, Lee AV, Oesterreich S. Baylor College of Medicine, Houston, TX.

177 **Defective Tumour Necrosis Factor-α (TNF-α) Production and Impaired TNF-α - Induced ICAM-1 Expression in BRCA1 Mutation Carriers.**
Budinsky AC, Wagner TMU, Kubista M, Wolfram RM, Kubista E, Brodowica T, Zielinski CC. Vienna, Austria.

178 **Cyclins, CDK-Inhibitors and Retinoblastoma Protein Aberrations in Breast Cancer.**
Lodén M, Hilmer Nielsen N, Emdin SO, Landberg GP. Umea University, Umea, Sweden.

179 **Analysis of Differential Downstream Signaling between Breast Cancer Cell Lines Overexpressing Epidermal Growth Factor Receptor and ErbB2.**
Siwak DR, Lopez-Berestein G, Tari AM. University of Texas M. D. Anderson Cancer Center, Houston, TX.

180 **HER-2/neu/ErbB-2 Status by Immunohistochemistry and FISH: Clonality and Progression with Recurrence and Metastases.**
Edgerton SM, Merkel DE, Moore DH, Thor AD. ENHRI/ Northwestern University, Evanston, IL; University of California, San Francisco, CA.

THURSDAY, DECEMBER 7

7:30-9:30 POSTER SESSION II & CONTINENTAL BREAKFAST

Supported by an educational grant from Aventis Oncology

Detection/Diagnosis

Detection

201 **Analysis of p43 Positive Lymphocytes from Peripheral Blood for Early Detection of Non Palpable Breast Cancer.**
Auerbach L, Hellan M, Alexander RC, Moroz C, Panzer S, Harald RR, Kubista E. University, Vienna, Austria; SMZ Ost, Vienna, Austria; Beilinson, Tel Aviv, Israel.

202 **Refined HER2/neu Diagnosis in Breast Cancer by Consecutive Immunohistochemistry and FISH.**
Buehler H, Evers K, Bangemann N, Kuhle A, Schaller G. University Hospital Benjamin Franklin, Berlin, Germany.

203 **FDG-PET for the Detection of Recurrent or Metastatic Breast Cancer.**
Kang HJ, Moon WK, Lee D-S, Chung J-K, Lee MC, Youn Y-K, Oh SK, Choe KJ, Noh D-Y. Seoul National University, College of Medicine, Seoul, Korea.

204 **Pilot Study of 0.5 Tesla Dedicated Magnetic Resonance Imaging for Early Detection of Breast Cancer in Young High Risk Women.**
Rubinstein WS, Sumkin JH, Poller W, Huerbin ML, Vogel VG. University of Pittsburgh, Pittsburgh, PA; Graduate School of Public Health, Pittsburgh, PA; Magee-Womens Hospital, Pittsburgh, PA.

205 **Early Detection of Breast Cancer: Who is Responsible?**
Fuentes-Alburo A, Chavez-MacGregor M, Ramirez-Ugalde MT, De la Garza-Salazar JG. Instituto Nacional de Cancerologia-Mexico, Mexico City, DF, Mexico.

Biopsy Techniques

206 **Cost Analysis of Stereotactic Vacuum Assisted Core Biopsy Vs Stereotactic Large Core Excisional Biopsy (Site Select) Vs Needle Localization Biopsy by Pathologic Outcome.**
Hughes KS, Smallman J, Germaine T, DeAngelis EA, Dedrick CG, Rolfs AT, Sites VR, Moskos MM, Sabo S, Robinson C. Lahey Clinic, Peabody, MA.

207 **An Evaluation of Complications of Stereotactic Vacuum Biopsy with the Mammotome of the Breast.**
Hahn M, Scheler P, Pollow B, Kuner RP, Fischer A, Hoffmann G. St Josefs-Hospital, Wiesbaden, Germany.

208 **Surgical Assessment of the Surrounding Breast and Axilla after a Tumor-Positive ABBI Biopsy.**
Schneider J, Lucas R, Rabadan F, Reillo A, Escalonilla A, Ruibal A, Tejerina A. Fundacion Tejerina-Centro de Patologia de la Mama, Madrid, Spain; Universidad del Pais Vasco, Bilbao, Spain; Fundacion Jimenez Diaz, Madrid, Spain.

Diagnostic Pathology

209 **Cytologic Diagnosis of Nipple Discharge by Intragalactophoric Aspiration Method.**
Hou M-F, Tsai K-B, Lin H-J, Chai C-Y, Liu C-S, Huang T-J, Huang C-J, Huang Y-S, Hsieh J-S, Chen F-M, Wang J-Y, Chan H-M, Chuang C-H, Ouyang F. Kaohsiung Medical University, Taiwan.

210 **Quantitative Immunohistochemistry with Antibodies to Phosphorylated Histone 3: A Novel Method to Identify Mitotic Cells in Tissue Sections.**
Stanton JJ, Coltrera MD, Yaziji H, Gown AM. University of Washington, Seattle, WA; PhenoPath Laboratories and IRIS, Seattle, WA.

211 **Squamous Cell Cancer - A Detailed Clinical, Pathologic and Molecular Assessment of 17 Cases.**
El-Maraghi RH, Verma S, Jabi M, Frenkel V. The University of Ottawa, Ottawa, ON; Ottawa Regional Cancer Center, Ottawa, ON, Canada.

212 **The Implications of Malignant Alterations in Cytoskeletal Proteins on Keratin-Based Analyses in Breast Cancer.**
Fuchs IB, Buehler H, Sehouli J, Lichtenegger W, Schaller G. Charité Campus Virchow Klinikum, Berlin, Germany; University Hospital Benjamin Franklin, Berlin, Germany.

213 **Infrared Spectroscopic Imaging: A New Tool for the Pathology Laboratory.**
Story GM, Marcott C, Lower EE, Yassin RS, Dukor RK. The Procter and Gamble Company, Cincinnati, OH; University of Cincinnati, Cincinnati, OH; Vysis, Inc., Downers Grove, IL.

214 **Radial Scars: Correlation of Morphology, Imaging and Tumor Markers.**
Kent SA, Wiley EL, DeLeon P, Adler YT. Northwestern University Medical School, Chicago, IL.

Epidemiology and Outreach

Epidemiology

215 **Measurement of Breast Pain: Significant Descriptors from a Modified Short McGill Pain Questionnaire.**
Khan SA, Apkarian AV. Upstate Medical University, Syracuse, NY.

Outreach/Education/Advocacy

216 **Emotional Disturbance at the Time of Breast Biopsy: Is This a Teachable Moment?**
O'Neill SM, Davison D, Rubinstein WS, Vogel VG. University of Pittsburgh Cancer Institute and Magee-Womens Hospital (UPCI/MWH), Pittsburgh, PA.

217 **Breast Cancer on the World Wide Web: Implications for Breast Cancer Specialists.**
Patel AR, Bradpiece HA, Morgan MW. St. Margaret's Hospital, Epping, Essex, United Kingdom.

218 **Impact of Consumers on Scientific Review of Breast Cancer Research Proposals.**
Andejeski Y, Bisceglio IC, Dickersin K, Johnson JE, Robinson SI, Smith HS, Visco FM, Rich I, and the USAMRMC Fiscal Year 1995 Breast Cancer Research Program Integration Panel. Department of Defense Congressionally Directed Medical Research Programs, Fort Detrick, MD.

219 **Some Reflections on Clinical Trials.**
Hetrick VR. You Are Not Alone, Los Angeles, CA.

220 **The Grassroots Fight to End Breast Cancer: Advocacy and Action.**
Warner MD. National Breast Cancer Coalition, Washington, DC.

Racial Aspects

221 **BRCA1 and BRCA2 Mutations in Breast Cancer Patients of African American Descent.**
Haffty BG, Alvarez-Franco M, Silber A, Matloff E, Bale AE. Yale University School of Medicine, New Haven, CT.

222 **Ethnic Differences in Estrogen Receptor Negativity, Tumor Stage, Grade and Histology in a Retrospective Breast Cancer Review.**
Butler WM, Cunningham JE, Reynolds JA, Sweatman CA, for the South Carolina Comprehensive Breast Center. South Carolina Cancer Center, Columbia, SC.

223 **Comparison of Breast Cancer Cases in the Arab-American and Caucasian Populations of Metropolitan Detroit.**
Do HT, Kau T-Y, Weiss LK, Severson RK, Schwartz KL. Karmanos Cancer Institute, Detroit, MI.

Treatment

Adjuvant Therapy

224 **Rate of Chemotherapy Related Amenorrhea Associated with Adjuvant Adriamycin and Cytoxan (AC) and Adriamycin and Cytoxan Followed by Taxol (AC+T) in Early Stage Breast Cancer.**
Stone ER, Slack RS, Novielli A, Ellis M, Baidas S, Gelmann E, Cohen P, Warren R, Stearns V, Hayes DF, Isaacs C. Georgetown University Hospital, Washington, DC.

225 **WITHDRAWN**

226 **Toxicity and Early Survival Results of a Prospective, Randomized Adjuvant Trial Comparing Toremifene and Tamoxifen in Node-Positive Breast Cancer.**
Holli K, Valavaara R, Blanco G, Kataja V, Hietanen P, Flander M, Pukkala E, Joensuu H. Tampere University Hospital, Tampere, Finland; Turku University Hospital, Turku, Finland; Oulu University Hospital, Oulu, Finland; Kuopio University Hospital, Kuopio, Finland; Helsinki University Hospital, Helsinki, Finland; South Karelia Central Hospital, Lappeenranta, Finland; Finnish Cancer Registry, Helsinki, Finland.

227 **Cognitive Changes and Menopausal Symptoms of Women Receiving Adjuvant Chemotherapy for Breast Cancer. Early Results of a Matched Cohort Study.**
Tchen N, Downie FP, Theriault M, Tannock IF. Princess Margaret Hospital, Toronto, ON, Canada.

228 **The European Table-Study-Group: Safety Data and Serum Hormone Levels of the Adjuvant Therapy in Premenopausal Breast Cancer Patients Comparing Leuprorelinacetate and CMF.**
Untch MM, Wallwiener DD, Schmid PP, Sobotta KK, Bondar GG, Kienle EE, Possinger KK. Ludwig-Maximilian-University, Munich, Germany; Humboldt-University, Berlin, Germany; Antitumor Centre of Donezk, Donezk, Ukraine; Takeda Pharma, Aachen, Germany; University, Tuebingen, Germany.

229 **Do Women with 4 or More Involved Axillary Nodes Need Taxol or Just More Adjuvant Chemotherapy?**
Cameron DA. Edinburgh University, Edinburgh, Scotland, United Kingdom.

230 **High Dose Epirubicin and Cyclophosphamide (EC) Vs Cyclophosphamide, Methotrexate, Fluorouracil (CMF) in High Risk Premenopausal Breast Cancer Patients: 5-Year Results of a Prospective Randomized Trial.**
Galligioni E, Cetto G, Crivellari D, Nascimben O, Buonadonna A, Molino A, Lucenti A, Graiff C, Barni S, Puccetti C, Ferrazzi E, Recaldin E, Sava C, Saracchini S, Sacco C, Talamini R, and the GOCNE (Gruppo Oncologico Cooperativo del Nord Est). Italy.

231 **Tumor Biological Factors uPA and PAI-1 as Stratification Criteria for Risk-Adapted Adjuvant Chemotherapy: Second Interim Analysis of a Randomized Multicenter Trial in Node-Negative Breast Cancer.**
Prechtl A, Thomssen C, Harbeck N, Meisner C, Braun M, Selbmann HK, Graeff H, Schmitt M, Jänicke F, and the German Chemo N0 Study Group. Frauenklinik der Technischen Universität, München, Germany; Universitätsfrauenklinik, Hamburg, Germany; Institut für Medizinische Statistik, Tübingen, Germany.

232 **The ATAC ('Arimidex', Tamoxifen, Alone or in Combination) Trial: The Effectiveness of Transvaginal Ultrasonography and Diagnostic Hysteroscopy in the Prediction of Endometrial Abnormalities in Asymptomatic Postmenopausal Women.**
Jackson TL, Duffy SRG, on Behalf of the ATAC Trialists Group (Endometrial Sub-Protocol). St James's University Hospital, Leeds, West Yorkshire, United Kingdom.

233 **The ATAC ('Arimidex', Tamoxifen, Alone or in Combination) Trial: Transvaginal Ultrasound Scan Findings Overestimate Observed Pathological Findings in Postmenopausal Gynaecologically Asymptomatic Women before Treatment.**
Jackson TL, Duffy SRG, on Behalf of the ATAC Trialists Group (Endometrial Sub-Protocol). St James's University Hospital, Leeds, West Yorkshire, United Kingdom.

234 **The Incidence of Neutropenic Events and Impact of Dose Intensity in the Adjuvant Treatment of Breast Cancer: The UK Breast Cancer Neutropenia Audit Group.**
Leonard RCF, Thomas R. University of Edinburgh, Edinburgh, United Kingdom; Addenbrooke's Hospital, Cambridge, United Kingdom.

235 **The Adjuvant Therapy of Breast Cancer: How to Translate Proportional Risk Reductions into Absolute 10-Year Benefits.**
Thome SD, Loprinzi CL. Mayo Clinic, Rochester, MN.

236 **Pharmacokinetics of 'Arimidex' and Tamoxifen Alone and in Combination in the ATAC Adjuvant Breast Cancer Trial.**
Dowsett M, on Behalf of the ATAC Trialists' Group. Royal Marsden Hospital, London, United Kingdom.

237 **Adjuvant Chemotherapy Vs No Further Treatment in High-Risk Node Negative Breast Cancer. Ten Year Results of a Prospective Randomized Trial.**
Milla A, Milla L, Rallo L, Solano V. Ntra. Sra. del Pilar Hospital, Barcelona, Spain.

238 **Feasibility of Full Dose Docetaxel (Taxotere™) after Dose-Intensive Doxorubicin (Adriamycin™) as Adjuvant Therapy for High-Risk Primary Breast Cancer.**
Ellis GK, Thompson T, Craig V, Rinn K, Gralow J, Livingston RL. University of Washington, Seattle, WA.

239 **Actually Applied Dose Intensity of Adjuvant Chemotherapy in Clinical Practice in Patients with Primary Breast Cancer.**
Jackisch C, Jaber M, Burkamp U, Roesel S, Raab G, Koch OM, Dame W, Gropp C, Gleumes L, Eiermann W, Schneider HPG. University Muenster, Muenster, Germany; Raphaelsklinik, Muenster, Germany; Paracelsusklinik, Osnabrueck, Germany; Staetisches Krankenhaus, Guetersloh, Germany; Frauenklinik vom Roten Kreuz, Munich, Germany; Krankenhaus Johanna-Etienne, Neuss, Germany.

240 **Adriamycin-Cyclophosphamide Adjuvant Chemotherapy Produced a 90% 5 Year Disease-Free Survival in 450 Women with Rapidly Growing Invasive Node Negative Breast Cancer.**
Jones SE, Clark G, Koleszar S, Ethington G, Mennel R, Kerr R, Pippen J, Blum J, Kitchens L, George T, Paulson R, Denham C, Stone M, Brooks B, Orr D. Texas Oncology, P.A. and the Sammons Cancer Center at Baylor University Medical Center, Dallas, TX.

241 **Suboptimal Dosing in Adjuvant Breast Cancer Chemotherapy: Evidence from a Nationwide Survey.**
Crawford J, Dale DC, Lyman GH, for the ANC Study Group. Duke University, Durham, NC; University of Washington, Seattle, WA; Albany Medical College, Albany, NY.

242 **Adjuvant FEC Polychemotherapy for Fast Proliferating Node Negative Breast Cancer Patients: A Randomized Clinical Trial.**
Paradiso AV, Schittulli F, Mangia A, Marzullo F, De Lena M. National Cancer Institute, Bari, Italy.

243 **Determinants of Outcome in Adjuvant Chemotherapy of Breast Cancer: Dose Intensity Vs. Total Dose Vs. Dose Size.**
Hryniuk WM, Peters WP, Ragaz J. Karmanos Cancer Institute, Detroit, MI; B.C. Cancer Agency, Vancouver, BC, Canada.

Neoadjuvant Therapy

244 **Improved Survival with Continuous Infusional ECisF over Conventional AC as Pre-Operative Chemotherapy for Early Breast Cancer.**
Smith IE, A'Hern RP, Howell A, Hickish T, O'Brien M, Mansi J, Wilson C, Robinson A, Pratt W, Price C, Perren T, Laing R, Jones A, Iveson T, Stein R, Gallagher C, Morgan J, on Behalf of TOPIC Trial Group. Royal Marsden, London, United Kingdom.

245 **Study of Differential Gene Expression before and after Preoperative Chemotherapy of Breast Cancer.**
Volz JO, Frühauf JH, Vielhauer S, Schneider J, Volz-Köster SR. University Hospital Mannheim, Mannheim, Germany.

246 **Remission Rate and Toxicity of Preoperative Therapy with Concurrent Application of Tamoxifen Together with Adriamycin and Docetaxel in Comparison to Chemotherapy Alone – Results of the Phase IIB – GEPARDO Trial.**
Blohmer JU, von Minckwitz G, Costa S, Raab GH, Eidtmann H, Hilfrich J, Jackisch C, Merkle E, Gademann G, Graf E, Tulusan AH, Kaufmann M, and the GEPARDO Study Group. GABG, Berlin, Germany.

247 **High Complete Pathological Response in Locally Advanced Breast Cancer Using Paclitaxel and Cisplatin.**
Ezzat AA, Ibrahim EM, Ajarim DS, Rahal MM, Raja A, Stuart RK, Tulbah AM, Kandil A, Al-Malik OA, Bazarbashi SN. King Faisal Specialist Hospital & Research Centre, Riyadh, Saudi Arabia.

248 **GEPARDUO - A Prospective Randomized Trial on Preoperative Chemotherapy in Operable Breast Cancer (T2-3,N0-2,M0) Comparing Dose Intensified (DI) Adriamcycin/ Docetaxel with Sequential Adriamycin/Cyclophosphamide Followed by Docetaxel - First Interim Analysis.**
Jackisch C, von Minckwitz G, Eidtmann H, Costa SD, Raab G, Blohmer JU, Schuette M, Gerber B, Merkle E, Gademann G, Lampe W, Hilfrich J, Tulusan AH, Graf E, Kaufmann M. University of Muenster, Muenster, Germany; University of Frankfurt/Main, Frankfurt/Main, Germany; University of Kiel, Kiel, Germany; Frauenklinik vom Roten Kreuz, Munich, Germany; University Berlin Charíté, Berlin, Germany; Bethesda Hospital, Essen, Germany; University of Rostock, Rostock, Germany; University of Halle, Halle, Germany.

249 **Relationship of Clinical and Pathologic Response to Neoadjuvant Chemotherapy and Outcome of Locally Advanced Breast Cancer.**
Gajdos C, Tartter PI, Bleiweiss IJ, Jaffer S, Estabrook A. The Mount Sinai Medical Center, New York, NY; St. Lukes-Roosevelt Hospital, New York, NY.

250 **Phase II Trial Combining Docetaxel and Doxorubicin in the Neoadjuvant Setting in Patients with Operable Breast Carcinoma: Final Results.**
Tubiana-Hulin M, Dieras V, Fumoleau P, Combe M, Misset J-L, Vannetzel J-M, Bachelot T, Lotz V, Ganem G. Centre R.Huguenin, St-Cloud; Institut Curie, Paris; Centre R.Gauducheau, St-Herblain; C.H. Le Mans; Hôpital Paul Brousse, Villejuif; Clinique Hartmann, Neuillys/Seine; Centre L. Bérard, Lyon; Laboratoire Aventis, Montrouge; Centre Jean Bernard, Le Mans, France.

251 **High Pathological Response Rate Induced by Primary Docetaxel Monotherapy in Operable Breast Cancer.**
Chollet P, Amat S, Penault-Llorca F, Fétissof F, Body G, Mouret-Reynier MA, Bons JM, Curé H, Dauplat J, Bougnoux P. Centre Jean Perrin and INSERM U484, Clermont-Ferrand; CHU Bretonneau, Tours, France.

252 **Phase II Study of Doxorubicin and Docetaxel as Neoadjuvant Therapy for Women with Stage IIB or III Breast Cancer.**
Limentani S, Erban J, Sprague K, Packman H, O'Leary M, Folatko C, Parma C. Carolinas Medical Center, Charlotte, NC; New England Medical Center, Boston, MA.

253 **Phase II Trial of Neoadjuvant Chemotherapy with Docetaxel and Doxorubicin, Surgery, Adjuvant CMF, and Radiotherapy +/- Tamoxifen in Locally Advanced Breast Cancer.**
Valero V, Esteva FJ, Sahin AA, Booser DJ, Strom EA, Esparza-Guerra LT, Ross MI, Rosales MF, Ibrahim NK, Cristofanilli M, Buchholz TA, Hunt KK, Hortobagyi GN. U. T. M. D. Anderson Cancer Center, Houston, TX.

254 **Neoadjuvant Chemotherapy with Taxotere-Epirubicin-5-Fluorouracil in Locoregionally Advanced Breast Cancer: Preliminary Report.**
Baltali E, Altundag MK, Abbasoglu O, Ozisik Y, Guler N, Atahan L. Hacettepe University, Ankara, Turkey.

255 **Phase II Trial of Modified Sequential CAF Regimen in Locally Advanced Breast Carcinoma: Safety and Efficacy Report.**
Valdivia S, Santillana S, Cotrina J, Gomez H, Abugattas J, Velarde R, Vigil C, Leon L, Flores C, Vallejos C. Instituto de Enfermedades Neoplasicas, Lima, Peru.

256 **Dose-Dense Preoperative Chemotherapy with Sequential Doxorubicin and Docetaxel for Operable and Inoperable Stage II-IIIB Breast Cancer.**
Tolnai E, Cooper B, Silverman P, Overmoyer B, Moss T. Case Western Reserve University, Cleveland, OH; Impath, Reseda, CA.

257 **Epirubicin/Cyclophosphamide High Dose as Primary Chemotherapy in Locally Advanced Breast Cancer (T2-T4/N0-N2/M0) – Preliminary Data/Toxicities.**
Dresel VC, Rinas N, Feltmann K, Stolte M, Tulusan AH. Klinikum Bayreuth, Bayreuth, Germany.

258 **Sequential Epirubicin-Paclitaxel Administration for Advanced Breast Cancer. A Randomized Phase I Trial.**
Focan C, Graas MP, Beauduin M, Canon JL, Salmon JP, Jerusalem G, Focan-Henrard D. Clin.Saint-Joseph, Liège, Hôp. Jolimont-Haine, St-Paul, Clin. Notre Dame, Charleroi, CH Peltzer-La Tourelle, Verviers, CHU Sart-Tilman, Liège, Belgium.

259 **The Correlation of Histone H3 Labeling Index and Post Chemotherapy PET Scan in Patients with LABC Treated with Neoadjuvant Doxorubicin and Docetaxel.**
Gupta-Burt S, Deshpande CG, Coon J, Sivaraman S, Preisler H, Ali A, Marcus E. Rush University, Chicago, IL; Cook County Hospital, Chicago, IL.

260 **A Phase II Neoadjuvant Trial of Sequential Doxorubicin and Docetaxel for the Treatment of Stage III Breast Cancer Measuring STAT Activation as a Predictor of Response to Therapy.**
Minton SE, Garcia R, Dalton W, Muro-Cacho C, Ku N, Cox C, Dupont E, Reintgen D, Shons A, Fields K, Sullivan D, Jove R. H. H. Lee Moffitt Cancer Center & Research Institute, University of South Florida, Tampa, FL.

261 **Neoadjuvant High-Dose Sequential Chemotherapy with Adriamycin, Paclitaxel, and Cyclophosphamide Is Feasible and Effective in Poor Prognostic Patients with Locally Advanced Breast Cancer.**
Rahal MM, Ezzat AA, Ibrahim EM, Ajarim DS, Raja A, Stuart RK, Tulbah AM, Kandil A, Bazarbashi SN. King Faisal Specialist Hospital & Research Center, Riyadh, Saudi Arabia.

262 **P-Glycoprotein Expression Is Not Induced by the First Dose of Neoadjuvant Paclitaxel Treatment, So Probably Does Not Stop the Initial Apoptotic Response in Non-Responsive Breast Cancers.**
Symmans WF, Yee HT, Volm MD, Demaria S, Chiriboga L, Shapiro RL, Kim AY, Muggia F. New York University Medical Center, New York, NY.

263 **Locally Advanced Primary Breast Cancer: Medium Term Results of a Randomised Trial of Multimodal Therapy Versus Initial Hormone Therapy.**
Tan S-M, Cheung KL, Willsher PC, Blamey RW, Chan SY, Robertson JFR. Nottingham City Hospital, Nottingham, United Kingdom.

264 **Gemzar, Adriamicyn and Taxol in Primary Chemotherapy in Breast Cancer: Preliminary Results.**
Sánchez-Rovira P, Dueñas B, González E, Jaén A, Dueñas R, Porras I, Gómez A, Medina B, Fernández M, Mohedano N, Martínez-Muro JL, Lozano A. Ciudad de Jaén Hospital, Jaén, Spain.

265 **Hemoglobin Values during Neoadjuvant Chemotherapy Using Epirubicin, Paclitaxel Followed by CMF. Results from a Randomized Multicenter-Trial.**
von Koch F, Kahlert S, Sobotta K, Crohns C, Konecny G, Bauerfeind I, Nestle-Kraemling C, Untch M. Ludwig-Maximilians-University, Munich, Germany.

Tumor Cell Biology

Antiestrogens

266 **Combination Antiestrogen/Antiprogestin Therapy of MCF-7 Breast Cancer Cells Inhibits Cell Proliferation through an Rb-Dependent Pathway.**
Schoenlein PV, Hou D, Hou M, Jones E, Kim I, Lewis J, Barrett J, Rackley D, Ogle T. Medical College of Georgia, Augusta, GA.

267 **Molecular Mechanism of Action of a New Clinically Relevant Antiestrogen (GW7604) Related to Tamoxifen.**
Bentrem DJ, Dardes RC, MacGregor-Shafer J, Zapf JW, Jordan VC. Northwestern University Medical School, Chicago, IL; Signal Pharmaceuticals, San Diego, CA.

268 **Estrogen-Like Action of the Tamoxifen-Estrogen Receptor Complex: A Mechanism for Drug Resistance in Breast Cancer.**
Jordan VC, Liu H, MacGregor-Shafer J, Lurie RH. Northwestern University, Chicago, IL.

269 **Effects of the Antiestrogens Tamoxifen and LY353381.HCl (Arzoxifene) on Endometrial Cancer Growth.**
Dardes RC, Bentrem DJ, O'Regan R, MacGregor-Shafer J, Jordan VC. Northwestern University Medical School, Chicago, IL.

270 Comparison of the Antiestrogenic and Estrogenic Activities of EM-652.HCl and Lasofoxifene in Human Endometrial Adenocarcinoma Ishikawa Cells and in the Ovariectomized Mouse Model.
Martel C, Gauthier S, Simard J, Mérand Y, Labrie F. Laval University Medical Center (CHUL) and Laval University, Québec, QC, Canada.

271 Antitumor Activity of Tamoxifen at Low Doses before Surgery.
Pigatto F, Veronesi P, Pelosi G, Bonanni B, Cazzaniga M, Guerrieri-Gonzaga A, Torrisi R, Arnone P, Bassi F, Robertson C, Decensi A. European Institute of Oncology, Milan, Italy.

272 Tamoxifen Effects on the Brain - Correlating Serial Positron Emission Tomography with Neurophysiologic Testing.
Mortimer JE, Donnelly J, Ball L, Knapp DL, Mintun M. Washington University, St. Louis, MO.

Endocrinology

273 Differential Effects of Steroidal Type I and Non-Steroidal Type II Anti-Aromatase Agents.
Miller W. Western General Hospital, Edinburgh, United Kingdom.

274 Paradoxical Effects of Aromatase Inhibitors: Increased Activity of Target Enzyme Following Prior Exposure to Some but Not All Aromatase Inhibitors.
Vidya R, Dixon JM, Evans DB, Miller WR. University of Edinburgh, Edinburgh, United Kingdom; Novartis, Basel, Switzerland.

275 Expression of a Novel Factor, com1, Is Regulated by Vitamin D in Breast Cancer Cells.
Ree AH, Bratland A, Risberg K, Maelandsmo GM, Fodstad O. Norwegian Radium Hospital, Oslo, Norway; Norwegian Radium Hospital, Oslo, Norway; University of Oslo, Oslo, Norway.

276 OC Use and Breast Cancer – Effect on Tumorbiology and Prognosis.
Schoenborn I, Möhner M, Lichtenegger W. Charite Campus Virchow, Humboldt-University, Berlin, Germany.

Estrogen Receptors

277 Estrogen Receptor Beta Is Expressed by Human Breast Fibroblasts.
Palmieri C, Saji S, Warner M, Gustaffson J-A, Coombes RC. Imperial College School of Medicine, London, United Kingdom; Karolinska Institutet, Novum, Huddinge, Sweden.

278 Association of Breast Cancer and Estrogen Receptor Gene Polymorphism.
Lueftner DI, Schweigert M, Roots I, Possinger K, Cascorbi I. Medizinische Klinik II, Berlin, Germany; Institut für Klinische Pharmakologie, Berlin, Germany.

279 Identification of a Novel Variant Estrogen Receptor Transcript in Human Breast Cancer.
Maaroufi Y, Lacroix M, Leclercq G. Laboratoire J-C Heuson de Cancérologie Mammaire, Institut Jules Bordet, Brussels, Belgium.

Genetics

280 Feasibility of Measuring Gene Expression Patterns Using Core Biopsies of Human Primary Breast Cancers and cDNA Microarray Technology.
Chang J, O'Connell P, Hilsenbeck SG. Baylor College of Medicine, Houston, TX.

9:30-10:00 PLENARY LECTURE I

EBCTCG: Worldwide Overview Results, Year 2000
Richard Peto, PhD
University of Oxford
Oxford, England, UK

10:00-12:00 GENERAL SESSION II

10:00 13. Zoladex™ (Goserelin) Vs. CMF as Adjuvant Therapy in Pre-/Perimenopausal Early (Node Positive) Breast Cancer: Preliminary Efficacy, QOL and BMD Results from the ZEBRA Study.
Jonat W, on Behalf of the ZEBRA (Zoladex Early Breast Cancer Research Association) Trialist's Group. University of Kiel, Germany.

10:15 14. A Randomized Double-Blind Multicenter Study of Pre-Operative Tamoxifen Versus Femara® (Letrozole) for Postmenopausal Women with ER and/or PgR Postive Breast Cancer Ineligible for Breast-Conserving Surgery. Correlation of Clinical Response with Tumor Gene Expression and Proliferation.
Ellis MJ, Jaenicke F, Llombart-Cussac A, Mauriac L, Vinholes J, Coop A, Singh B, Dugan M, Evans DB, Chaudri HA, Borgs M, and the Letrozole Neoadjuvant Breast Cancer Study Group. Duke University, Durham, NC; UKE, Hamburg, Germany; Instituto Valenciano de Oncologia, Valencia, Spain; Institut Bergonie, Bordeaux, France; Santa Casa de Porto Alegre, Porto Alegre, Brazil; Georgetown University, Washington, DC; Novartis Pharmaceuticals, East Hanover, NJ; Novartis Pharma AG, Basel, Switzerland.

10:30 15. Distinct Deletions in the *egfr* Gene Enhancing Transcription Force Tumor Growth in Breast Carcinogenesis.
Brandt BH, Boecker A, Tidow N, Rust S, Nakachi K, Schmidt H, Boecker W, Buerger H. Institute for Clinical Chemistry, Muenster, Germany; Gerhard-Domagk.Inst., Muenster, Germany; Saitama Cancer Center Research Inst., Saitama, Japan.

10:45 16. Cytogenetic Differences Revealed by Comparative Genomic Hybridization (CGH) in Japanese and German Breast Cancer Cases.
Buerger H, Brandt B, Nakachi K, Dockhorn-Dworniczak B, Boecker W. University of Muenster, Muenster, Germany; Saitama Cancer Center, Saitama, Japan.

11:00 17. Detection of Pre-Malignant and Malignant Breast Cells by Ductal Lavage.
Dooley WC, Veronesi U, Elledge R, O'Shaughnessy J, Ljung BM, et al. Johns Hopkins, Istituto Europeo di Oncologia, Baylor Houston, Baylor Dallas, UCSF, et al.

11:15 18. Fiberoptic Ductoscopy for Patients with Nipple Discharge.
Shen K-W, Shen Z-Z, Nguyen M, Barsky SH, Shao Z-M. Cancer Hospital, Shanghai Medical University, Shanghai, China; UCLA School of Medicine, Los Angeles, CA.

11:30 19. FDG-PET and Lymphoscintigraphy: Towards a Comprehensive Evaluation of the Internal Mammary Lymph Nodes.
Bellon JR, Byrd DR, Dunnwald LK, Eary JF, Anderson BO, Mankoff DA. University of Washington, Seattle, WA.

11:45 20. Prostate Epithelium-Derived Ets Transcription Factor Is a Candidate Breast Tumor Marker and a Breast Tumor Antigen.
Ghadersohi A, Sood AK. Roswell Park Cancer Institute, Buffalo, NY.

12:00-12:55 LUNCH [Ticket Required]

1:00-2:00 CASE PROBLEMS IN PRIMARY BREAST CANCER

2:00-2:30 PLENARY LECTURE II

Studying Alternative Therapies for Patients with Breast Cancer
Charles L. Loprinzi, MD
Mayo Clinic
Rochester, Minnesota

2:30-5:00 MINI-SYMPOSIUM II

ER Structure, Function, and Clinical Utility
Suzanne AW Fuqua, PhD, Moderator
Baylor College of Medicine, Houston, Texas

2:30 **Introduction**

2:30 **ERα and ERβ: The Yin-Yang Principle of Estrogen Action**
Jan-Åke Gustafsson, MD PhD
Karolinska Institute
Stockholm, Sweden

3:00 **ER Signaling Into the Cyclin/CDK/Rb Pathway**
Robert L. Sutherland, PhD
Garvan Institute of Medical Research
Sydney, Australia

3:30 **ER Structure and Function Relationship**
Geoffrey L. Greene, PhD
University of Chicago
Chicago, Illinois

4:00 **The Molecular Pharmacology of SERMs: Implications for the Treatment and Prevention of Breast Cancer**
Donald P. McDonnell, PhD
Duke University Medical Center
Durham, North Carolina

4:30 **Validation of ER and PgR by Immunohistochemistry in Breast Cancer: Where Are We?**
D. Craig Allred, MD
Baylor College of Medicine
Houston, Texas

Supported by an educational grant from AstraZeneca

5:00-6:30 OPEN

6:30-8:30 THE BRINKER INTERNATIONAL AWARDS DINNER

The Susan G. Komen Breast Cancer Foundation and the 23rd Annual San Antonio Breast Cancer Symposium cordially invite you to join us in the Marriott Rivercenter ballroom for dinner and presentation of the Brinker International Awards for Breast Cancer Research. [Ticket Required]

FRIDAY, DECEMBER 8

7:30-9:30 POSTER SESSION III & CONTINENTAL BREAKFAST

Prognosis and Response Predictions

Predictive Factors

301 **Can Her-2/Neu Select Patients to Primary CMF?**
Falo C, Moreno A, Lloveras B, Prieto L, Figueras A, Escobedo A. Institut Català d'Oncologia, Barcelona, Spain.

302 **Clinical-Pathologic Assessment of the Clinical Sensitivity to Single Agent Taxane Therapy for Metastatic Breast Cancer.**
Van Poznak C, Tan L, Panageas K, Arroyo C, Hudis C, Norton L, Seidman AD. Memorial Sloan Kettering Cancer Center, New York, NY.

303 **Evaluation of Predictive Factors in a Randomized Trial of Preoperative Dose-Intensified Adriamycin-Docetaxel +/- Tamoxifen in Primary Operable Breast Cancer.**
von Minckwitz G, Sinn HP, Raab G, Blohmer JU, Graf E, Kaufmann M, for the GABG. Goethe-University, Frankfurt, Germany.

304 **Can Changes in Proliferation Predict Response to Tamoxifen in Breast Cancer?**
Iqbal S, Anderson TJ, Marson L, Dixon JM, Miller WR. Western General Hospital, Edinburgh, Scotland, United Kingdom.

305 **N-Terminally Truncated HER-2 Protein, p95, Is Associated with Lymph Node Metastasis.**
Ramsey BE, Keenan EJ, Sexton G, Clinton GM. Oregon Health Sciences University, Portland, OR.

306 **Choice of Chromogen Can Affect Determination of HER2 Status by Immunohistochemistry.**
Dressler LG, Cowan D, Geradts J, Wang WY, Moore D, Little D, Miller A, Vick C, Newman B, Millikan R. University of North Carolina, Chapel Hill, NC.

307 **Lack of Interaction between Tumor Histologic Grade and Response to Chemotherapy in Node Negative and ER Negative Breast Cancer.**
Park K, Yothers G, Bryant J, Wolmark N, Paik S. NSABP, Pittsburgh, PA.

308 **Self Organizing Maps and Prognosis of Advanced Breast Cancer Patients with Bone Metastases Receiving Letrozole or MA.**
Schmid P, Wischnewsky MB, Possinger K. Charite, Berlin, Germany; University, Bremen, Germany.

309 **Amplification of Topoisomerase IIa or c-erbB-2 Predicts Response to Doxorubicin and Docetaxel in Locally Advanced Breast Cancer.**
Coon JC, Marcus E, Gupta-Burt S, Seelig S, Jacobson K, Fronda G, Preisler H. Rush University, Chicago, IL; Cook County Hospital, Chicago, IL; Vysis, Inc., Downers Grove, IL.

310 **Response Assessment by HER2/neu Status in a Trial with Dose-Intensified Weekly Paclitaxel in Metastatic Breast Cancer.**
Lueftner DI, Akrivakis C, Flath B, Wernecke K-D, Possinger K. Universitätsklinikum Charité, Campus Mitte, Berlin, Germany; Universitätsklinikum Charité, Campus Virchow-Klinikum, Berlin, Germany.

311 **Immunomarkers on Cell-Blocks from Fine-Needle Cytopuncture as Predictors of Tumor Response to Preoperative Chemotherapy in Non-Metastatic Primary Breast Carcinoma.**
Briffod M, Cohen-Solal C, Hacène K, Le Doussal V, Tubiana-Hulin M. Centre René Huguenin de Lutte contre le Cancer, Saint-Cloud, France.

312 Immunohistochemical Variation of Human Equilibrative Nucleoside Transporter 1 Protein in Human Primary Breast Cancers.
Mackey JR, Jennings L, Dabbagh L, Vsianska M, Koski S, Young JD, Coupland R, Cass CE. University of Alberta and Cross Cancer Institute, Edmonton, Alberta, Canada; Masaryk Memorial Cancer Institute, Brno, Czech Republic.

313 Prospective Analysis of the Oxygenation of Malignant Breast Tumors as a Predictor of Response to Primary Systemic Chemotherapy.
Raab GH, Auer F, Scheich D, Feldmann HJ, Molls M, Eiermann W. Red Cross Hospital, Munich, Germany; Technical University, Munich, Germany.

Treatment

Chemotherapy - General

314 6 Cycles of Epirubicin®/Taxotere® Versus 6 Cycles of 5FU/ Epirubicin®/Cyclophosphamide (FEC) as First Line Metastatic Breast Cancer Treatment: Preliminary Results of a Randomized Phase II Trial.
Dieras V, Tubiana-Hulin M, Bougnoux P, Bonneterre M-E, Mayer F, Delozier T, Culine S, Dohollou N, Samak R, Suissa J, Bonneterre J. Institut Curie, Paris; Centre R Huguenin, Saint-Cloud; CHU Bretonneau, Tours; Centre O.Lambret, Lille; Centre F.Leclerc, Dijon; Centre F.Baclesse, Caen; Centre Val D'Aurelle, Montpellier; Polyclinique Bordeaux Nord; Clinique St George, Nice; Laboratoire Aventis, Montrouge, France.

315 A Phase II Study of Weekly Docetaxel as Primary Chemotherapy in Stage II and III Breast Cancer: Preliminary Results.
Estévez L, Cuevas JM, Antón A, Florián J, Lopez-Vega JM, Velasco A, and the Breast Cancer Investigation Spanish Group. GEICAM, Spain.

316 Weekly Taxol and Carboplatin Regimen in Patients with Advanced Breast Cancer: A Phase II Study.
Loesch D, Robert N, Asmar L, Gregurich MA, Dakhil S. Oncology Hematology Associates, Inc. - South, Indianapolis, IN.

317 Phase II Trial of Weekly Docetaxel (Taxotere) Alone or in Combination with Trastuzumab (Herceptin) in Patients with Metastatic Breast Cancer.
Sparano JA, Malik U, Manalo J, Rajdev L, Sarta C, Hopkins U, Fineberg S. Montefiore Medical Center, Bronx, NY.

318 Phase I-II Trial of Pegylated Liposomal Doxorubicin (Doxil) Plus Docetaxel (Taxotere) in Patients with Advanced Breast Cancer.
Sparano JA, Malik U, Wolff A, Rajdev L, Sarta C, Hopkins U. Montefiore Medical Center, Bronx, NY; Winship Cancer Center, Atlanta, GA.

319 Randomized Trial of Dose-Intense Adjuvant Chemotherapy with Epirubicin and Cyclophosphamide in High-Risk Breast Cancer Patients.
Thomssen C, Untch M, Behrens K, Kahlert S, Sattler D, Oberlechner E, Kuhn W, Lebeau A, Dettmer P, Konecny G, Jaenicke F. University Hospital, Hamburg; University Hospital Großhadern, Munich; Technical University, Munich; Hospital Landshut, Germany.

320 Oral Single-Agent Estramustine Is Active in Advanced Breast Cancer after Failure with Anthracyclines and Taxanes.
Zelek L, Barthier S, Riofrio M, Sevin D, Spielmann M. Institut G.-Roussy, Villejuif, France.

321 Phase II Trials of Carboplatin/Docetaxel and Carboplatin/ Docetaxel/Traztuzumab as First Line Therapy for Metastatic Breast Cancer.
Brufsky A, Lebish J, Shanahan C, Dyky M-A, Jacobs S, Stoller R, Baar J, Kim H, Kane K, Belani C. University of Pittsburgh Cancer Institute, Pittsburgh, PA.

322 Phase II Study of Gemcitabine and Cisplatin in Patients with Metastatic Breast Cancer and Failure on Prior Chemotherapy: A North Central Cancer Treatment Group Trial.
Burch PA, Mailliard JA, Hillman DW, Perez EA, Krook JE, Rowland KM, Ingle JN. Mayo Clinic, Rochester, MN; Missouri Valley Cancer Consortium CCOP, Omaha, NE; Mayo Clinic, Jacksonville, FL; Duluth CCOP, Duluth, MN; Carle Cancer Center CCOP, Urbana, IL.

323 Low Weekly Dose of Paclitaxel in the Treatment of Metastatic Breast Cancer Patients. A Phase II Study.
Colozza M, Mosconi AM, Gori S, Cherubini R, Basurto C, Tonato M. Policlinico Hospital, Perugia, PG, Italy.

324 Monthly Cisplatin and Gemcitabine as Second Line Chemotherapy for Patients with Advanced Breast Cancer.
Galvez CA, Galmarini F, Curie M. Hosp. Municipal de Oncología; Bs.Aires, Argentina.

325 Paclitaxel Maintenance Treatment Following First Line Chemotherapy with Anthracyclines Plus Paclitaxel in Metastatic Breast Cancer: Preliminary Results from the Italian MANTA 1 Study.
Gennari A, Manzione L, Del Mastro L, Amadori D, De Lena M, Moretti G, Grifalchi F, Valsecchi R, Luzi Fedeli S, Carrara B, Conte P. St. Chiara Hospital, Pisa, Italy; S.Carlo Hosp., Potenza, Italy; Pierantoni Hosp., Forlì, Italy; IRCCS, Bari, Italy; Spallanzani Hosp, Reggio Emilia, Italy; La Sapienza University, Rome, Italy; S.Carlo Borromeo Hosp., Milan, Italy; S.Salvatore Hosp., Pesaro, Italy; IST, Genova, Italy.

326 Weekly Docetaxel in Advanced Breast Cancer with Progression after Treatment with Anthracyclines.
González la Puente CC, Morales S, Méndez M, Baena JM, Borrega P, Centellas M, Puerto-Pica JM, Milla A, Galán A, Castellanos J, Lorenzo A, Palombo A, González Barón M. H. El Tomillar, Sevilla; H. Arnau de Vilanova, Lerida; H. de Mostoles, Madrid; H. Puerta del Mar, Cádiz; H. San Pedro de Alcántara, Cáceres; H. Sagrado Corazón, Barcelona; H. Infanta Cristina, Badajoz; Nª Sª del Pilar, Barcelona; H. Gral Sagunto, Valencia; H. Xeral Cies, Vigo; H. Puerto Real, Cádiz; Clin Nª Sª de os Remedios, Barcelona; H. La Paz, Madrid, Spain.

327 Results of Two Open-Label Multicentre Pilot Phase II Trials with Herceptin® in Combination with Docetaxel and Platinum Salts (Cis- or Carboplatin) as Therapy for Advanced Breast Cancer in Women Overexpressing HER2.
Nabholtz JM, Crown J, Yonemoto L, Tannenbaum S, Klimo P, Patel R, Fumoleau P, Sanchez J, Prady C, Villa D, Ellis E, Pegram M, Lindsay MA, Slamon D, and the Breast Cancer International Research Group (BCIRG). UCLA, Los Angeles, CA.

328 Taxotere and Adriamicin-Cyclophosphamide (AC) in High Risk Localized Breast Cancer.
Pérez MM, Oakinin A, Velasco A, Jiménez U, Donnay O, Pérez Carrión R. H. Princesa, Madrid, Spain.

329 Docetaxel and Doxorubicin as First-Line Chemotherapy for Metastatic Breast Cancer after Adjuvant CMF Failure. A Multicenter Nonrandomized Study.
Pienkowski T, Gruszfeld AI, Foszczynska-Kloda M, Zaluski J, Utracka-Hutka B. Memorial Cancer Center, Warsaw, Poland; Regional Cancer Center, Szczecin, Poland; Regional Cancer Center, Poznan, Poland; Cancer Center, Gliwice, Poland.

330 A Pilot Study of Docetaxel and Vinorelbine in Metastatic Breast Cancer.
Pienkowski T, Gruszfeld AI. Memorial Cancer Center, Warsaw, Poland.

331 **Capecitabine: An Active and Well Tolerated Treatment Option for Patients with Metastatic Breast Cancer Recurring after Taxane-Containing Chemotherapy. Results of a Multicenter Phase II Trial.**
Reichardt P, von Minckwitz G, Lück HJ, Thuss-Patience PC, Jonat W, Kölbl H, Kiebak D, Kuhn W, Schindler AE, Jänicke F, Mohrmann S, Floemer F, Frings S. Charité, Humboldt-Universität, Berlin, Germany; Universitäts-Frauenklinik, Frankfurt, Germany; Medizinische Hochschule, Hannover, Germany; Universitätsklinikum, Kiel, Germany; Martin-Luther-Universität, Halle, Germany; Universitäts-Frauenklinik, Freiburg, Germany; Technische Universität, München, Germany; Universitätsklinikum, Essen, Germany; Universitätskrankenhaus, Hamburg, Germany; Heinrich-Heine-Universität, Düsseldorf, Germany; Hoffmann-La Roche AG, Grenzach-Wyhlen, Germany; Hoffmann-La Roche Inc., Nutley, NJ.

332 **Phase II Study of Weekly Docetaxel in the Treatment of Metastatic Breast Cancer.**
Aihara T, Kim Y, Takatsuka Y. Kansai Rosai Hospital, Amagasaki, Hyogo, Japan.

333 **Weekly Combination of Taxol, 5-Fluorouracil and Leucovorin in Advanced Breast Cancer Patients.**
D'Ottavio AM, Nisticò C, Valenza R, Frontini L, Barni S, Carnino F, Vaccaro A, Garufi C, Zappalà A, Aschelter AM, Tropea F, Izzo F, Terzoli E. Regina Elena Cancer Institute, Rome, Italy; Maurizio Ascoli Hospital, Palermo, Italy; S. Paolo Hospital, Milan, Italy; S. Gerardo Hospital, Monza, Italy; S. Anna Hospital, Torino, Italy.

334 **Capecitabine Named Patient Programme for Patients with Advanced Breast Cancer: The UK Experience.**
Leonard RCF, Anderson A, Twelves C, Hutcheon A, Bissett D, Chaturvedi A, Rowland C, Mansi J, Chan S, Carmichael J, on Behalf of the UK Capecitabine Audit Group. Western General Hospital, Edinburgh; Beatson Oncology Centre, Glasgow; Aberdeen Royal Infirmary, Aberdeen, United Kingdom.

335 **Taxane-Based Chemotherapy-Induced Colitis in Breast Cancer Patients: M. D. Anderson Cancer Center Experience.**
Li Z, Wang M, Mante RM, Ibrahim NK. U. T. M. D. Anderson Cancer Center, Houston, TX.

336 **Advanced Breast Cancer: Combination Chemotherapy with Doxorubicin and Weekly Docetaxel Treatment.**
Méndez M, Quiben R, López P, Enrech S, Menéndez P, Sancho JF, Casinello J, Domínguez S, Palomero MI, Perez-Manga G. H. de Móstoles, Madrid, H. Gómez Ulla, Madrid, H. Guadalajara; Txagorritxu, Vitoria, H. Gregorio Marañón, Madrid, Spain.

337 **Epirubicin-Docetaxel Administered Simultaneously as First-Line Chemotherapy in Metastatic Breast Cancer.**
Morales S, Castellanos J, Lorenzo A, González A, Méndez M, Ramos M, Casal J, Belón J, Lizón J, Frau A, Moreno-Nogueira JA, Domínguez S. Spanish Breast Cancer Hospitals, Spain.

338 **Effectiveness of Topotecan as a Primary Chemotherapy of Brain Metastases in Patients with Breast Cancer.**
Oberhoff C, Hilfrich J, Kieback DG, Mesrogli M, Sehouli J, von Minckwitz G, von Soest C, Wuerstlein R, Deertz H, Staab HJ, Schindler AE, for the TOPBRAIN-Study Group.

339 **Advanced Inflammatory Breast Cancer: Combination Chemotherapy with Doxorubicin and Weekly Docetaxel Treatment.**
Quiben R, Méndez M, López P, Enrech S, Menéndez P, Sancho JF, Casinello J, Palomero MI, Perez-Manga G. H. de Móstoles, Madrid, H. Gómez Ulla, Madrid, H. Guadalajara; H. Gregorio Marañón, Madrid, Spain.

340 **Weekly Docetaxel in Advanced Anthracycline-Resistant Breast Cancer.**
Ramos M, González A, Amenedo M, González Quintas A, Gamazo JL, Losada G. Centro de Oncología de Galicia, Coruña, Hospital de Povisa, Vigo, Spain.

341 **Effects of Adjuvant Anthracyclines on the Activity of Epirubicin Containing First Line Chemotherapy in Metastatic Breast Cancer.**
Salvadori B, Gennari A, Landucci E, Rondini M, Orlandini C, Donati S, Conte P. S. Chiara Hospital, Pisa, Italy.

342 **Schedule-Dependency of Antitumor Activity in Combination Therapy with Capecitabine/Doxifluridine and Docetaxel in Breast Cancer Models.**
Tominaga T, Ouchi K, Tanaka Y, and the Docetaxel/Doxifluridine Trial Group. Tokyo, Japan; Nippon Roche Research Center, Kamakura, Kanagawa, Japan.

343 **Reduction of Neurotoxicity from Schedule Modification of Dose-Dense Paclitaxel.**
Green MC, Buzdar AU, Wingate AD, Hortobagyi GN. M.D. Anderson Cancer Center, Houston, TX.

344 **Dose-Dense Doxetaxel and Mitoxantrone Is a Promising Chemotherapy in Patients with High Risk Metastatic Breast Cancer.**
König E, Kurbacher C, Rein D, Mallmann P. University of Cologne, Cologne, Germany.

345 **Dose-Finding Study of UFT Modulated by Leucovorin in Combination with Weekly Paclitaxel in Patients with Metastatic Breast Cancer.**
Lebedinsky CA, Breier SM, Ayaviri C, Cot C, Salvatori JM. Hospital Israelita, Capital Federal, Buenos Aires, Argentina.

346 **Neoadjuvant Chemotherapy with Cyclophosphamide, Methotrexate, 5-FU, Prednisone, Doxorubicin, and Vincristine with G-CSF, Followed by Paclitaxel Plus Cisplatin, as Adjuvant Chemotherapy in Stage II and III Breast Cancer.**
Martín JI, Adell A, Estévez LG, Dómine M, León A, Casado V, Castillo M, Lobo F. Fundación Jiménez Díaz, Madrid, Spain.

347 **A Multicentric Phase II Trial of Gemcitabine Plus Epirubicin Plus Taxol (GET) as 1st Line Chemotherapy for Metastatic Breast Cancer.**
Mazzoni F, Donati S, Gennari A, Cetto G, Crino L, Galligioni E, Lucenti A, Mansutti M, Galligioni E, Molino AM, Tumolo S, Conte PF. Bellaria Hospital, Bologna, Italy; Santa Chiara Hospital, Pisa, Italy; Osp. Civile Maggiore, Verona, Italy; Santa Chiara Hospital, Trento, Italy; Santa Maria Hosp., Udine, Italy; Santa Maria Hosp., Pordenone, Italy.

348 **Cardiac Troponin I in Breast Cancer Patients Receiving Anthracycline Chemotherapy.**
Bauer-Kosinska B, Miskiewicz Z, Pienkowski T, Kaminska L. Memorial Cancer Center, Warszawa, Poland.

349 **A Phase II Trial of Escalated Dose Docetaxel with G-CSF Support in Patients with Advanced Breast Cancer.**
Mitchell P, Basser R, Harris M, Ng S, Gibbs P, Chipman M, Grigg A, Jeffrey A, James R, Gargano J, Riva A, Appia F, Green M. Austin & Repatriation Medical Centre, Heidelberg West, VIC, Australia; Royal Melbourne Hospital, Parkville, VIC, Australia; Western Hospital, Footscary, VIC, Australia; Aventis Research & Development, Melbourne and Paris.

350 **Dolastatin-10 in Patients with Advanced Breast Cancer, an NCCTG Study.**
Perez EA, Fishkin PA, Hillman DW, Krook JE, Kaur J, Hynes HE, Ingle JN. Mayo Foundation, Rochester, MN; Illinois Oncology Research Association CCOP, Peoria, IL; Duluth CCOP, Duluth, MN; Wichita Community Clinical Oncology Program, Wichita, KS.

351 **A Phase II Study of Oxaliplatin and 5-Fluorouracil in Advanced/Metastatic Breast Carcinoma Patients Previously Treated with Taxanes: Preliminary Results.**
Spielmann M, Chouaki N, Cottu P, Zelek L, Vannetzel J, Misset J, Dieras V, Lebail N, Marty M. Inst. Gustave Roussy, Villejuif; CAC, Kremlin; Hôp. St Louis, Paris; Clinique Hartman, Neuilly s/ Seine; Hôp. Paul Brousse, Villejuif; Inst. Curie, Paris; Sanofi-Synthelabo, France.

Chemotherapy - High Dose

352 **A Phase I Study of Sequential Dose Dense Induction Chemotherapy with High Dose Consolidation for the Treatment of High-Risk Primary Breast Cancer.**
Emens LA, Kennedy MJ, Fetting JH, Davidson NE, Armstrong DA. The Johns Hopkins Oncology Center, Baltimore, MD.

353 **Influence of CD34+ Cell Dose and Tumor Cell Contamination of Leukapheresis Products on Posttransplant Outcome in 76 Patients with Metastatic Breast Cancer.**
Hensel M, Schneeweiss A, Egerer G, Hohaus S, Grischke E-M, Bastert G, Ho AD. University of Heidelberg, Heidelberg, Germany.

354 **Dose of Mobilization Chemotherapy Is a Significant Predictor of Outcome for Hormone Receptor Negative Patients Treated with High Dose Cyclophosphamide, Carboplatin and Thiotepa (CTCb) and Peripheral Blood Progenitor Cells.**
Sysel IA, Birch R, Wood JM, Schwartzberg LS, West WH. Response Oncology Inc. Memphis, TN.

Chemotherapy - Support

355 **Once-Per-Cycle Pegylated Filgrastim (SD/01) Is as Effective and Safe as Daily Filgrastim in Reducing Chemotherapy-Induced Neutropenia over Multiple Cycles of Therapy.**
Holmes FA, Jones SE, O'Shaughnessy J, Vukelja S, George T, Khandelwal P, Savin M, Kirby R, Hyman W, Mcintyre K, Melnyk A Jr., Huslig R, Richards D, Glaspy J, Meza L, Dhami M, Budman DR, Hill RL, Neumann T, Brassard M, Yang B-B, Schwab G, Liang BC. US Oncology, Dallas, TX; Amgen Inc., Thousand Oaks, CA; UCLA, Los Angeles, CA; Southwest Oncology Associates, Lafayette, LA; Eastern Connecticut Hematology/Oncology Associates, Norwich, CT; Northshore University Hospital, Manhasset, NY.

356 **Early Recognition of Taxane-Associated Canaliculitis Important in Preserving Lacrimal Duct Function.**
Kneuper Hall R, Metzner-Sadurski JK, Marshall M, Howard GR. Medical University of South Carolina, Charleston, SC.

Other Therapies

357 **Phase I Clinical Trial of Mammary Ductoscopy in Patients with Pathologic Nipple Discharge.**
Dietz JR, Kim JA, Dawson A, Grundfest SF, Crowe JP. Cleveland Clinic Foundation, Cleveland, OH.

358 **Comparison of Oral Versus Parenteral Administered Bisphosphonates in Breast Cancer Patients with Bone Metastases**
Untch M, Blokh E, Marschner N, Kindler M, Lange OF, Konecny G, Golan Ch, Hurtz H-J, Diel IJ. Ludwig-Maximilians-University, Munich, Germany; University, Heidelberg, Germany; Clinic of Turmorbiology, Freiburg, Germany.

359 **Prinomastat Inhibits Primary Tumor Growth and Retards Osteolytic Disease in Xenograft Models of Breast Cancer Metastasis.**
Waltham M, Tester A, Ruangpanit N, Bills M, Shalinsky DR, Thompson EW. St. Vincent's Institute of Medical Research, Melbourne, Australia; Agouron Pharmaceuticals, Inc., San Diego, CA.

360 **A Phase II Study with Lutrin® for Recurrent Cutaneous Breast Cancer: A Redox Active Photosensitizer with a Dual Mechanism of Action That Potentiates Response to PDT and Chemotherapy.**
Phan S-C, Carlson R, Lustig R, Kaplan M, Renschler M, Magda D. Pharmacyclics, Inc., Sunnyvale, CA; Stanford University, Stanford, CA; University of Pennsylvania, Philadelphia, PA; Albert Einstein Medical Center, Philadelphia, PA.

361 **Use of Bisphosphonates in Breast Cancer Metastatic to Bone at a Regional Cancer Centre.**
Dhesy-Thind SK, Goffin JR, Reyno L, Major PP. Hamilton Regional Cancer Centre, Hamilton, ON, Canada.

362 **Primary Lymphoma of the Breast: A Retrospective Analysis of 11 Cases.**
Kuemmel S, Krocker J, Budner M, Breitbach G, Kohls A, Possinger K, Michniewicz K, Elling D. OZK, Berlin, Germany; Stralsund, Germany; Neunkirchen, Germany; Ludwigsfelde, Germany; Charitè Campus Mitte, Berlin, Germany.

DCIS

363 **WITHDRAWN**

364 **Breast Conserving Therapy of Ductal Carcinoma In Situ (DCIS): Size of the Lesion Is the Main Risk Factor for Residual Tumor.**
Decker T, Obenaus R, Kettritz U, Ruhnke M, Schmidt D, Smolarek-Roterberg K, Morack G, Schneider W. The Breast Unit, Berlin-Buch Medical Center, Berlin, Germany.

365 **Rural-Urban Differences in Radiation Therapy for Ductal Carcinoma In Situ (DCIS) of the Breast.**
Schootman M, Aft R. Washington University School of Medicine, Saint Louis, MO.

366 **Avoiding Radiotherapy Following Breast Conserving Surgery for Ductal Carcinoma In Situ of the Female Breast.**
Choy CWK, Hu JCC, Kirkpatrick KL, Wells C, Perry N, Mokbel K, Carpenter R. St Bartholomew's Hospital, London, United Kingdom.

367 **Dilemmas in the Management of DCIS: A Survey of Patterns of Care in the United Kingdom.**
Ross GM, Landau D, Hall E, Sainsbury R. Royal Marsden Hospital, London, United Kingdom; Institute of Cancer Research, London, United Kingdom; Huddersfield Royal Infirmary, Yorks., United Kingdom.

Patient Management

368 **Using a Risk-For-Distress Measure to Predict Risk for Treatment Refusal.**
Hryniuk WM, Hryniuk L, Palen E, Du W, Darga L, Mood D. Karmanos Cancer Institute, Detroit, MI.

369 **Rapid Bone Loss after Chemotherapy in Breast Cancer Patients.**
Batista N, Oramas J, Rodriguez E, Cruz J, Llanos M, Rodriguez L-M, Gomez A, Gonzalez-Reimers E, Santolaria F. Hospital Universitario de Canarias, Universidad de La Laguna, La Laguna, Tenerife, Spain.

370 **First Report of a Comprehensive Survey of Chronic Arm Morbidity after Curative Breast Cancer Treatment – Incidence and Its Impact on Quality of Life.**
Kwan W, Weir L, Olivotto I, Dingee C, McGregor GP, Jackson J. Fraser Valley Cancer Centre, Surrey, BC, Canada; Vancouver Cancer Centre, Vancouver, BC, Canada; BC Cancer Agency, Vancouver, BC, Canada.

371 **Metastases to the Breast: Determinants of Survival.**
Wills DD, Meric F, Mirza NQ, Singletary SE, Ames FC, Kuerer HM, Ross MI, Newman LA, Feig BW, Pollock RE, Hunt KK. M. D. Anderson Cancer Center, Houston, TX.

Tumor Cell Biology

Antigens and Markers

372 CD34, CD117 and Actin Expression in Phylloides Tumor of Breast.
Hsieh HF, Liu CT, Chen CM, Chang CL, Shyu JS, Chen CL, Ham HJ. Yee-Zen General Hospital, Taoyuan, Taiwan; Armed Forces Taoyuan General Hospital, Taoyuan, Taiwan; National Defense Medical Center, Taipei, Taiwan.

Carcinogenesis

373 Overexpression of HIF-1α in Breast Carcinogenesis.
Bos R, Zhong H, Semenza GL, Pinedo HM, Simons JW, van Diest PJ, van der Wall E. Free University Hospital, Amsterdam, The Netherlands; Emory University School of Medicine, Atlanta, GA; The Johns Hopkins University School of Medicine, Baltimore, MD.

Apoptosis

374 Hydroxymethylacylfulvene (HMAF, MGI 114, Irofulven) Induces Apoptosis in the Caspase-3 Deficient Breast Cancer Cell MCF-7.
Herzig MCS, Liang H, Woynarowska B, Woynarowski JM. Cancer Therapy and Research Center, San Antonio, TX.

375 CP461 Induces Apoptosis and Growth Inhibition of Breast Cancer Cells Independent of HER2/neu Receptor Expression.
Liu L, Lloyd M, Pegram MD, Slamon DJ, Pamukcu R, Thompson WJ. Cell Pathways Inc., Horsham, PA; UCLA School of Medicine, Los Angeles, CA.

376 Treatment with the Pure Antiestrogen Faslodex (ICI 182780) Increases the Sensitivity of MCF-7 Breast Cancer Cells Against Fas Induced Apoptosis.
Diel P, Loeffek S, Smolnikar K, Michna H. DSHS, Cologne, Germany.

377 Measurement of Apoptosis in Breast Cancer by Immunohistochemistry Using an Antibody Against the Active Form of Caspase 3 and Correlation with Tumour Histopathological Features
Hadjiloucas I, Gilmore AP, Bundred NJ, Streuli CH. University of Manchester, Manchester, United Kingdom.

378 Exisulind Inhibits Cell Growth, Induces Apoptosis, and Has Synergy with Herceptin and Taxotere in Breast Cancer Cells.
Pegram MD, Liu L, Lloyd M, Pamukcu R, Slamon DJ, Thompson WJ. UCLA School of Medicine, Los Angeles, CA; Cell Pathways, Inc., Horsham, PA.

379 Induction of Apoptosis in Breast Cancer Cells by Inhibition of Glucose Metabolism.
Zhang F, Aft RA. Washington University School of Medicine and John Cochran Veterans Administration Hospital, St. Louis, MO.

380 mRNA Expression, Localization and Genomic DNA Amplification of Decoy Receptor 3 (DcR3) in Breast Cancer.
Koyama Y, Kanbayashi C, Kaibe T, Kanda T, Tomita Y, Hayashi M, Sakurai K, Uemura M, Sato N, Hatakeyama K. Niigata University School of Medicine Faculty, Niigata, Japan.

9:30-10:00 PLENARY LECTURE III

Safe Viral Vectors Provide the Opportunity to Initiate Clinical Trials
C. Thomas Caskey, MD
Cogene BioTech Ventures
Houston, Texas

10:00-12:00 GENERAL SESSION III

10:00 21. Amplification of Topoisomerase II Alpha Is a Strong Predictor of Response to Epirubicin-Based Chemotherapy in HER-2/neu-Positive Metastatic Breast Cancer.
Isola JJ, Tanner M, Holli K, Joensuu H. Univ Tampere, Tampere, Finland; Helsinki University Hospital, Helsinki, Finland.

10:15 22. Transcription Factor YB-1 Predicts Clinical Drug Resistance and Patient Prognosis in Breast Cancer.
Harbeck N, Janz M, Dettmar P, Berger U, Schmitt M, Royer H-D. Technische Universität München, Munich, Germany; Max-Delbrück-Centrum für Molekulare Medizin, Berlin, Germany.

10:30 23. HER2-Status Predicts Complete Pathological Response in Primary, Operable Breast Cancer Treated with Neoadjuvant Epirubicin/Docetaxel + G-CSF.
Steger GG, Wenzel C, Schmidinger MP, Rudas M, Taucher S, Gnant MF, Jakesz R. University of Vienna, Vienna, Austria.

10:45 24. Phase II Trial of Herceptin Induction Followed by Combination Therapy with Paclitaxel and Carboplatin: A Minnie Pearl Research Network Trial.
Burris HA III, Hainsworth JD, Miranda FT, Broome CM, Houston GA, Jones SF, Erland J, Sullivan T, Greco FA. The Sarah Cannon Cancer Center and Tennessee Oncology, Nashville, TN; Northern Virginia Oncology Group, Fairfax, VA; Jackson Oncology Associates, Jackson, MS.

11:00 25. Treatment Beyond Progression in the Herceptin Pivotal Combination Chemotherapy Trial.
Tripathy D, Slamon D, Leyland-Jones B, Wolter J, Murphy M, Shak S, Stewart S. Univ. of California San Francisco, San Francisco, CA; Univ. of California Los Angeles, Los Angeles, CA; McGill Univ., Montreal, ON, Canada; Rush Presbyterian St. Lukes Medical Center, Chicago, IL; Genentech, Inc., S. San Francisco, CA.

11:15 26. Effects of ZD 1839 (Iressa™), a Novel EGF Receptor Tyrosine Kinase Inhibitor, on Breast Cancer Cell Proliferation and Invasiveness.
Anderson NG, Ahmad T, Chan KC, Bundred NJ. University of Manchester, Manchester, United Kingdom.

11:30 27. Zoledronic Acid Reduces Skeletal Related Events in Patients with Osteolytic Metastases: A Double-Blind, Randomized Dose Response Study.
Berenson J, Rosen L, Howell A, Porter L, Coleman R, Morley W, Dreicer R, Kuross S, Lipton A, Seaman J. West LA VA Medical Center, Los Angeles, CA; UCLA Medical Center, Los Angeles, CA; Christie Hospital, Withington, Manchester, United Kingdom; Dial Research Associates, Nashville, TN; Weston Park Hospital, Sheffield, United Kingdom; American Medical Research, Atlanta, GA; University of Iowa, Iowa City, IA; Duluth Clinic, Duluth, MN; Hershey Medical Center, Hershey, PA; Novartis Pharmaceuticals, East Hanover, NJ.

11:45 28. Clinical Activity with the Farnesyl Transferase Inhibitor R115777 in Patients with Advanced Breast Cancer – Relationship with Tumour Phenotype.
Johnston SRD, Hickish T, Ellis PA, Houston S, Howes AJ, Dowsett M, Palmer P. Royal Marsden Hosp., London, United Kingdom; Royal Bournemouth Hosp., Bournemouth; Kings College Hosp., London; Royal Surrey Hosp., Guilford; Janssen Research Foundation, Beerse, Belgium.

12:00-11:55 LUNCH [Ticket Required]

1:00-2:00 CASE PROBLEMS IN ADVANCED BREAST CANCER

2:00-2:30 PLENARY LECTURE IV

HRT and Breast Cancer
Ronald K. Ross, MD
Norris Comprehensive Cancer Center
Los Angeles, California

2:30-5:00 GENERAL SESSION IV

2:30 29. Morphometric and Cytogenetic Investigations in *In Situ* Carcinomas of the Breast.
Buerger H, Mommers EC, Littmann R, Diallo R, Poremba C, Dockhorn-Dworniczak B, Van Diest PJ, Boecker W. University of Muenster, Muenster, Germany; Free University Hospital, Amsterdam, The Netherlands.

2:45 30. Ductal Invasive G2 and G3 Carcinomas of the Breast Are the End Stage of at Least Two Different Lines of Genetic Evolution.
Buerger H, Mommers EC, Littmann R, Simon R, Diallo R, Dockhorn-Dworniczak B, van Diest PJ, Boecker W. University of Muenster, Muenster, Germany; Free University Hospital, Amsterdam, The Netherlands.

3:00 31. A Hypersensitive Estrogen Receptor α Protein in Premalignant Breast Lesions.
Hopp TA, Hilsenbeck S, Mohsin S, O'Connell P, Allred DC, Fuqua SAW. Baylor College of Medicine, Houston, TX.

3:15 32. Inhibition of Breast Cancer Cell Migration by Insulin-Like Growth Factor Binding Protein-1 (IGFBP-1).
Zhang X, Hartell JS, Gross JM, Sachdev D, Yee D. University of Minnesota Cancer Center, Minneapolis, MN.

3:30 33. TGF-β Stimulates Tumor Production of PTHrP Via Smad and MAP Kinase Signaling Pathways.
Kakonen S-M, Chirgwin JM, Selander KS, Grubbs BG, Yin JJ, Guise TA. UTHSCSA, San Antonio, TX; University of Turku, Turku, Finland.

3:45 34. Antigen Presentation Prevents Tumor Development in Tumor Challenged Her-2 Transgenic Mice.
Jabrane-Ferrat N, Campbell M, Peterlin M, Esserman L. UCSF, San Francisco, CA.

4:00 35. Risk of Ipsilateral Recurrence and Metachronous Contralateral Breast Cancer after Breast Conservation Therapy in Women with Germline BRCA1 or BRCA2 Mutations.
Robson M, Roberge D, Satagopan J, Chappuis P, Boyd J, Offit K, Foulkes W. Memorial Sloan-Kettering Cancer Center, New York, NY; Sir M.B. Davis-Jewish General Hospital, McGill University, Montreal, QC, Canada.

4:15 36. A Randomised Study of Sector Resection with and without Radiotherapy for Women with Node-Negative Stage I and II Breast Cancer.
Malmström P, Wallgren A, Andersson H, Holmberg L, Ingvar C, Jönsson P-E, Mattson J, Tennvall-Nittby L, Svensson JH, and the Swedish Breast Cancer Group. Lund University Hospital, Lund, Sweden.

4:30 37. Regional Nodal Failure Patterns Following Mastectomy without Radiation.
Katz A, Strom EA, Buchholz TA, Jhingran A, Theriault R, Singletary SE, McNeese MD. M.D. Anderson Cancer Center, Houston, TX.

4:45 38. Loco-Regional Radiation for High Risk Breast Cancer – Results of Short Fractionation.
Fairchild AM, Weir LM, Mates D, Olivotto IA. Queens University, Kingston, Canada; British Columbia Cancer Agency, Vancouver, Canada.

5:00-7:00 POSTER SESSION IV & RECEPTION

Detection/Diagnosis

Marrow and Blood Micrometastases

401 Prognostic Impact of Mucin-Positive Cells in the Bone Marrow of 1338 Patients with Primary Breast Cancer.
Diel IJ, Solomayer EF, Hahn M, Gollan CH, Schütz F, Bastert G. University Hospital, Heidelberg, Germany.

402 Real Time Monitoring of the Efficacy of Adjuvant Therapy in Breast Cancer Quantifying the Reduction of Circulating Tumor Cells by MAINTRAC (Laser Scanning Cytometry of Magnetic Bead Enriched Cells).
Pachmann K, Tolkmitt M, Mengel M, Rinas N, Lobodasch K, Tulusan AH, Pachmann U. TZB Bayreuth, Germany; DRK Chemnitz Rabenstein, Chemnitz, Germany; Klinikum Bayreuth, Bayreuth, Germany.

403 The Fate of Occult Metastatic Cells in Follow-Up Bone Marrow Aspirations of Patients with Primary Breast Cancer.
Janni WJ, Rjosk D, Hepp F, Kentenich C, Braun S. LMU Munich, Munich, Germany.

404 MHC Class I Expression of Metastatic Breast Cancer Cells Isolated from Peripheral Blood.
Pham DT, Mosca PJ, Clay TM, Morse MA, Vredenburgh JJ, Ross AA, Lyerly HK. Duke University Medical Center, Durham, NC; Nexell Therapeutics Inc., Irvine, CA.

405 Detection of Breast Cancer Cells in Bone Marrow and Peripheral Blood with Magnetic Bead Enrichment and Laser Scanning Cytometry (MAINTRAC).
Rinas N, Pachmann U, Pachmann K, Diel I, Tulusan AH. Klinikum Bayreuth, Bayreuth, Germany; Laboratory for Immunohematology and Gendiagnostic, Bayreuth, Germany; University of Heidelberg, Heidelberg, Germany.

406 An Ultra-Sensitive Tumor Enriched Immunocytochemical Assay for Detection of Micrometastases in Blood of Breast Cancer Patients.
Umiel T, George S, Joyce R, Moss TJ. IMPATH/BIS, Reseda, CA; Cancer and Blood Institute of the Desert, Rancho Mirage, CA; Baptist Medical Center, Jacksonville, FL.

Circulating Markers

407 Biochemical Markers of Bone Metabolism Predict Prognosis in Patients with Bone Metastases of Breast Cancer.
Takahashi S, Yoshida N, Koizumi M, Horikoshi N, Ogata E. Japanese Foundation for Cancer Research, Tokyo, Japan.

408 Independent Prognostic Role of Changes in CA 15-3 Serum Level and Clinical Response after First Line Chemotherapy in Metastatic Breast Cancer Patients.
Berruti A, Tampellini M, Gorzegno G, Danese S, Durando A, De Matteis A, Genta F, De Fabiani E, Nuzzo F, Manzin E, Sarobba MG, Castiglione F, Moro G, Giardina G, Farris A, Massobrio M, Dogliotti L, and the EPI-LON Group. Orbassano, Torino, Italy.

409 Serial Monitoring of Serum HER-2/neu in Women with Metastatic Breast Cancer Correlates with the Clinical Course of Disease.
Carney WP, Hamer PJ, Tenney DY, Johnson KA, Allard WJ, Yeung K, Neumann R, Brown-Shimer S. Oncogene Science/ Bayer Diagnostics, Cambridge, MA.

410 The Use of Blood Tumour Markers in the Monitoring of Metastatic Breast Cancer Unassessable for Response to Systemic Therapy.
Cheung KL, Evans AJ, Chan SY, Robertson JF. City Hospital, Nottingham, United Kingdom.

411 **Identification of Serum Nuclear Matrix Protein Markers of Breast Cancer Using Surface Enhanced Laser Desorption-Ionization (SELDI) Mass Spectroscopy.**
Watkins B, Szaro R, Matczak E, Zowczak M, Torlinksi L, Wu Y. Matritech Inc., Newton, MA; Harvard University, Boston, MA; K. Marcinkowski University of Medical Sciences, Poznan, Poland.

412 **Correlation of Shed Serum HER-2/neu and Tumor Marker CA 15-3 with Response to Chemotherapy for Metastatic Breast Cancer.**
Mueller V, Witzel I, Kuehnel P, Lueck H, Pantel K, Jaenicke F, Thomssen C, and the AGO-Breast Cancer Cooperative Group. University Hospital, Hamburg, Germany.

413 **Serum Levels of Circulating Intercellular Adhesion Molecule-1 (s-ICAM-1) in Patients with Breast Cancer before and after Surgery: Correlation with Pathological Parameters.**
Stravoravdi P, Sahpazidou D, Voyatzi S, Toliou T, Pavlidou E, Bousoulegas A. Theagenio Cancer Hospital, Thessaloniki, Greece.

Prognosis and Response Predictions

Prognostic and Predictive Factors - Methods

414 **Histologic Grade and ER/PgR Status in Breast Carcinoma.**
Hill KA, Wiley EL, Badve S. Northwestern University, Chicago, IL.

415 **HER2 Overexpression in Breast Cancer: Correlation between Quantitative Expression Data and Clinical Diagnostic Tests.**
Bartlett JMS, Going JJ, Watters AD, Mallon EA, Reeves JR, Richmond J, Donald B, Ferrier R, Cooke TG. University of Glasgow, Glasgow, United Kingdom; GRI, Glasgow, United Kingdom; Western Infirmary, Glasgow, United Kingdom.

416 **Comparison of HER-2/neu Analysis Using FISH and IHC When HercepTest® Is Scored Using Conventional Microscopy and Image Analysis.**
Bloom K, de la Torre-Bueno J, Press M, Gown A, Bauer K, Harrington D. Rush-Presbyterian St. Luke's Medical Center, Chicago, IL; ChromaVision Medical Systems, Inc., San Juan Capistrano, CA; Univ. of So. CA, Los Angeles, CA; PhenoPath Labs, Seattle, WA.

417 **HER-2 Copy Number or HER-2:Chromosome 17 Ratio – Which Gives the More Accurate Measure of Overexpression?**
Watters AD, Going JJ, Mallon EA, Reeves JR, Cooke TG, Bartlett JMS. University of Glasgow, Glasgow, United Kingdom; GRI, Glasgow, United Kingdom; Western Infirmary, Glasgow, United Kingdom.

418 **A Sequential Double Label Immunohistochemical Technique for the Simultaneous Evaluation of Nuclear and Membrane Markers in DCIS and Invasive Breast Cancer.**
Cowan DW, Dressler LG. University of North Carolina, Chapel Hill, NC.

419 **Chromogenic In Situ Hybridization (CISH): A Practical New Alternative to FISH to Detect HER-2/neu Amplification in Archival Breast Cancer Samples.**
Tanner MM, Gancberg D, DiLeo A, Larsimont D, Rouas G, Piccart M, Isola JJ. Univ Tampere, Tampere, Finland; Jules Bordet Institute, Bruxelles, Belgium.

420 **Analysis of the Potential Contribution of the Estrogen Receptor (ER) β in the ER Cytosolic Protein Assay of Breast Cancer.**
Brouillet J-P, Dujardin MA, Chalbos D, Rey JM, Grenier J, Maudelonde T, Pujol P. Hôpital A. de Villeneuve, Montpellier, France; Parc Euromédecine, Montpellier, France.

421 **Can Gene Expression Pattern Analysis Predict Recurrence in Node-Negative Breast Cancer?**
Immaneni A, Li Z, Hilsenbeck SG, Allred DC, O'Connell P. Baylor College of Medicine, Houston, TX.

422 **HER2/*neu* Alterations in Breast Cancer: A Comparative Study of Immunohistochemistry Using Three Antibodies and Quantitative PCR.**
O'Malley FP, Parkes R, Latta E, Tzan S, Zadro T, Arneson N, Mueller R, Blackstein M, Andrulis I. Mount Sinai Hospital; Samuel Lunenfeld Research Institute; University of Toronto, Toronto, ON, Canada.

423 **Time to Non-Breast Metastasis for Node Negative Breast Cancer Patients Who Received No Adjuvant Therapy.**
Chapman JW, Fish EB, Link MA. Sunnybrook and Womens College Health Sciences Centre, University of Toronto, Toronto, ON, Canada.

424 **Angiogenesis Index Is Associated with Early Recurrence in Patients Presenting with Primary Breast Cancer.**
Ellis RJ, Kimler BF, Fabian CJ, Tawfik O, Mehta RS, Kysthoobayeva A, Fruehauf JP. University of Kansas Medical Center, Kansas City, KS; Oncotech, Inc., Irvine, CA; University of California, Irvine, CA.

425 **Differential Event Forecasting and Improved Risk Assessment in Breast Cancer Using Neural Networks.**
Kates RE, Schmitt M, Harbeck N. Technische Universitaet Muenchen, Munich, Germany.

426 **Evaluation of an Automated System for Scoring Immunohistochemical Staining of HER2 in Breast Carcinoma Using Two Antibodies to HER2.**
Witton CJ, Going JJ, Mallon EA, Cooke TG, Bartlett JMS. Glasgow Royal Infirmary, Glasgow, United Kingdom; Western Infirmary, Glasgow, United Kingdom.

427 **Alterations of the Luteal Heat Cycle in Cancer-Associated Breasts.**
Hayes L, Wilson P, Affen J, Greenhalgh R, Cooley J, Evans DG, Tetlow L, Howell A. University Hospital of South Manchester, Manchester, United Kingdom.

Treatment

Breast Conservation

428 **Long-Term Complications Associated with Breast Conservation Surgery and Radiation Therapy.**
Meric F, Buchholz TA, Mirza NQ, Vlastos G, Singletary SE, Ross MI, Ames FC, Pollock RE, Feig BW, Kuerer H, Newman LA, Perkins GH, Strom EA, McNeese MD, Hortobagyi GN, Hunt KK. The University of Texas M. D. Anderson Cancer Center, Houston, TX.

429 **Breast Conserving Therapy in the Elderly: An Appropriate Treatment Choice.**
Vlastos G, Mirza NQ, Meric F, Hunt KK, Newman LA, Kuerer HM, Ames FC, Ross MI, Pollock RE, Buchholz TA, Hortobagyi GN, Singletary SE. The University of Texas M. D. Anderson Cancer Center, Houston, TX.

430 **Incidence and Survival Impact of Non-Breast Second Primary Malignancies Following Breast Conserving Therapy.**
Mirza NQ, Vlastos G, Meric F, Buchholz TA, Singletary SE, Ames FC, Newman LA, Kuerer HM, Feig BW, Ross MI, Pollock RE, Hortobagyi GN, Hunt KK. The University of Texas M. D. Anderson Cancer Center, Houston, TX.

431 **Preliminary Report of Ultrasound-Guided CryoAblation of Breast Tumors Using a 2.4-mm Probe.**
Caleffi M, Borghetti K, Antoniazzi R, Graudenz M, Duarte Filho D. Clinica de Mastologia, Porto Alegre, Brazil; Instituto de Patologia, Porto Alegre, Brazil; Serdil Radiologia, Porto Alegre, Brazil.

432 **Uncontrolled Local Disease after Salvage Treatment for Ipsilateral Breast Tumor Recurrence.**
Dalberg K, Liedberg A, Johansson U, Rutqvist LE. Mälarsjukhuset and Karolinska Hospital; Södersjukhuset, Stockholm, Sweden.

433 **Bilateral Reduction Mammoplasty to Improve Breast Conservation Therapy Results in Breast Cancer Patients with Macromastia.**
Newman LA, Kuerer HM, Hunt KK, Vlastos G, Gurtner J, Ames FC, McNeese MD, Robb GL, Singletary SE. M.D. Anderson Cancer Center, Houston, TX.

434 **Three-Dimensional Reconstruction of the Whole Breast Duct-Lobular Units Using Computer Graphics; With Special Reference to Ductal Anastomoses between Individual Duct-Lobular Units.**
Ohtake T, Kimijima I, Fukushima T, Takenoshita S. Fukushima Medical University School of Medicine, Fukushima, Japan.

Radiation Therapy

435 **Positive Margins Following Surgical Excision of Breast Carcinoma: Analysis of Pathologic Correlates.**
Miller AR, Guilherme B, Thomas PJ, Tif S, Morton KS, Cruz AB, Itien Y. University of Texas Health Science Center, San Antonio, TX.

436 **A Prospective Comparison of 3D Versus 2D Radiotherapy Treatment Planning of Left-Sided Breast Cancer Patients.**
Hardenbergh PH, Light KL, Zhou SM, Bentel GC, Marks LB. Duke University Medical Center, Durham, NC.

437 **Adjuvant Hypofractionated Conformal Radiation to the Tumor Bed in Selected Post-Menopausal Women with T1 Breast Cancers: A Pilot-Feasibility Study.**
Formenti SC, Joszef G. New York University, New York, NY; University of Southern California, Los Angeles, CA.

438 **The Evaluation of Myocardial Perfusion after Adjuvant Radiotherapy in Left-Sided Breast Cancer Patients.**
Niwinska A, Galecki J, Pienkowski T, Olszewska M, Kaniewska J. Memorial Cancer Centre, Warsaw, Poland.

439 **The Assessment of the Toxicity of Adjuvant Postmastectomy Chemoradiotherapy in Breast Cancer Patients.**
Niwinska A, Pienkowski T, Miskiewicz Z, Stelmaszczyk P. Memorial Cancer Centre, Warsaw, Poland.

440 **Accuracy of Ultrasound in Localization of Breast Boost Field.**
Ringash J, Whelan T, Elliott E, Minuk T, Sanders K. Princess Margaret Hospital and University of Toronto, Toronto, ON, Canada; Hamilton Regional Cancer Centre and McMaster University, Hamilton, ON, Canada; Hamilton Health Sciences Corporation and McMaster University, Hamilton, ON, Canada.

Surgery

441 **Factors Influencing Surgical Choices in Women with Breast Cancer.**
Staradub VL, Rademaker AW, Clauson J, Langerman A, Morrow M. Northwestern University Medical School, Chicago, IL.

442 **Surgical Morbidity and Patient Satisfaction Following Immediate Breast Reconstruction.**
Choy CWK, Kirkpatrick KL, Hu JCC, Mostafa A, Gattuso J, Mokbel K, Denton S, Carpenter R. St Bartholomew's Hospital, London, United Kingdom.

Hormone Replacement Therapy

443 **The Effects of Prolonged HRT Treatment in Normal Post-Menopausal Breast Epithelium.**
Dobson RRH, Chan CK, Knox F, Potten CS, Bundred NJ. Paterson Institute for Cancer Research, Christie Hospital NHS Trust, Manchester, United Kingdom.

444 **WITHDRAWN**

445 **Hormone Receptor Status and S-Phase in Breast Cancers – Relation to Use of Hormone Replacement Therapy.**
Isaksson E, Mahlman M, von Schoultz E. Karolinska Hospital, Stockholm, Sweden.

Cost-Effectiveness

446 **Use of HercepTest in Metastatic Breast Cancer Patients for Assignment to Herceptin: A Cost-Effectiveness Analysis.**
Elkin EB, Weinstein MC, Winer EP, Kuntz KM, Weeks JC. Harvard University, Cambridge, MA; Harvard School of Public Health, Boston, MA; Dana Farber Cancer Institute, Boston, MA.

447 **A Micro-Costing Analysis of Pamidronate/Zoledronic Acid Administration.**
Bajwa K, Markle J, Zacker C, Schulman K. Duke University, Durham, NC; Novartis Pharmaceuticals, East Hanover, NJ.

Tumor Cell Biology

Growth Factors/Inhibitors

448 **Inhibition of AP-1 Suppresses the In Vitro and In Vivo Growth of MCF-7 Breast Cancer.**
Liu Y, Ludes-Meyers J, Munoz-Medellin D, Kim HT, Zhang Y, Ge G, Schiff R, Osborne CK, Brown PH. Baylor College of Medicine, Houston, TX; Univ of Texas HSC, San Antonio, TX.

449 **Overexpression of the Parathyroid Hormone-Related Protein Receptor Increases the Mitogenic Responsiveness of MCF-7 Breast Carcinoma Cells.**
Anderson NG, Hoey RP, Ahmad T, Linforth R, Bundred NJ. University of Manchester, Manchester, United Kingdom.

450 **Stromal Insulin-Like Growth Factor 2 Is a Favorable Prognostic Marker for Breast Cancer, Modified by Age, Estrogen Receptor and Mannose 6-Phosphate/Insulin-Like Growth Factor 2 Receptor Status.**
Ellis MJ, Rasmussen A, DaCosta SA, Warren A, Trock B, Cullen KJ. Lombardi Cancer Center, Washington, DC.

451 **Growth Factor Regulation of the Forkhead Transcription Factor FKHR in Human Breast Cancer.**
Jackson JG, Yee D, Powell DR, Barr FG, Brattain MG. University of Texas Health Science Center at San Antonio, San Antonio, TX; University of Minnesota Cancer Center, Minneapolis, MN; Lexicon Genetics, The Woodlands, TX; University of Pennsylvania School of Medicine, Philadelphia, PA.

452 **The HGF/SF Antagonist, NK4, Suppressed HGF/SF-Induced Matrix Adhesion, Invasion and Phosphorylation of Paxillin in Human Cancer Cell Lines.**
Parr C, Davies G, Hiscox S, Nakamura T, Matsumoto K, Jiang WG, Mansel RE. University of Wales College of Medicine, Cardiff, United Kingdom; University of Osaka Medical School, Osaka, Japan.

453 **Peptide Suppression of Breast Cancer Growth: In Search of Mechanisms by Identification of Cellular Targets.**
Dauphinée MJ, Mizejewski GJ. Rumbaugh-Goodwin Institute for Cancer Research, Inc., Plantation, FL.

454 Loss of HER-2-Overexpression during Herceptin®-Therapy in Metastatic Breast Cancer.
Boettcher B, Kahlert S, Bauerfeind I, Nestle-Krämling C, Konecny G, Untch M. Ludwig-Maximilians-University, Munich, Germany.

455 Identification of AP-1 Regulated Genes in Human Breast Cancer Cells.
Kim H-T, Liu Y, Munoz-Medellin D, Brown PH. Baylor College of Medicine, Houston, TX.

Metastasis/Invasion

456 Imaging of Primary Tumors in Whole Animals Using Laser-Based Tomography.
Wyckoff JB, Frohlich V, Jones JG, Segall JE, Condeelis JS. Albert Einstein College of Medicine, Bronx, NY; University of Texas Health Science Center, San Antonio, TX.

457 Analysis of a Metastasis Gene on Chromosome 14q.
Martin MD, Osborne CK, Mohsin SK, Allred DC, O'Connell P. Baylor College of Medicine, Houston, TX.

458 KGF-Induced Gene Expression in MCF-7 Cells Using cDNA Expression Arrays.
Zang X, Learner ML, Brackett DJ, Pento JT. University of Oklahoma HSC, Oklahoma City, OK; VA Medical Ctr., Oklahoma City, OK.

459 The Transfection or Induction of Keratin 18 in MDA-MB 231 Breast Cancer Cells Results in Redifferentiation and Strongly Reduced Malignancy of the Clones.
Buehler H, Becker C, Fuchs I, Schaller G. University Hospital Benjamin Franklin, Berlin, Germany; University Hospital Charite, Berlin, Germany; Harvard Medical School, Boston, MA.

460 NK4, a HGF/SF Antagonist, Inhibits Breast Cancer Cell Invasion of Endothelium: Its Role in HGF/SF Induced Changes of Tight Junction Function.
Martin TA, Matsumoto K, Nakamura T, Mansel RE, Jiang WG, and the Metastasis Research Group. University of Wales College of Medicine, Cardiff, Wales, United Kingdom.

461 The Rate-Limiting Step in Metastasis: In Vivo Analysis of Intravasation at the Primary Tumor.
Wyckoff JB, Bailly M, Jones JG, Condeelis JS, Segall JE. Albert Einstein College of Medicine, Bronx, NY.

462 A Multi-Organ Metastatic Orthotopic Patient ER/PR-Positive Breast Cancer Nude Mouse Model.
Rashidi B, An Z, Wang X, Moossa AR, Hoffman RM. AntiCancer, Inc., San Diego, CA; University of California, San Diego, CA.

463 A New Murine Syngeneic Model of Breast Cancer Metastasis to Bone Expressing PTHrP.
Jacobs L, Watson SA, Morris TM, Robertson JF. University of Nottingham, Nottingham, United Kingdom.

464 Palpation Abets Breast Carcinoma Dissemination in the Setting of Lymphovascular Invasion.
Tomlinson JS, Kasraeian S, Barsky SH. UCLA School of Medicine, Los Angeles, CA.

465 Gemcitabine Completely Suppressed the Stimulating Effects of G-CSF on Clonal Proliferation and Migration of Cancer Cell Lines *In Vitro*.
Fritz J, Schmid P, Flath B, Becker M, Possinger K, Elstner E. Charité Campus Mitte, Humboldt-University, Berlin, Germany.

466 The Role of Caveolin-1 Gene Expression in Progression and Metastasis of Human Breast Cancer.
Lee H-R, Kim J, Ahn K-S, Kim M-K, Nam S-J, Yang J-H, Park K, Yoon S-S. Samsung Medical Center, SungKyunKwan University, School of Medicine, Seoul, Korea.

Immunology and Immunotherapy

467 Analysis of Type 1 and Type 2 T Cells in Breast Cancer by Intracellular Cytokine Staining and Flow Cytometry.
Campbell MJ, Scott J, Esserman LJ, Maecker H. UCSF, San Francisco, CA; Becton Dickinson, San Jose, CA.

468 Cellular Immune Responses to Immunodominant HER-2/neu Helper Peptides in Patients with Ductal Carcinoma In Situ (DCIS).
Gillogly MA, Sahin AA, Ioannides CG, Murray JL. UT M.D. Anderson Cancer Center, Houston, TX.

469 Elevated Serum Levels of Soluble ICAM-1 Observed in Patients with Breast Cancer Do Not Interfere with Anti-Her-2/neu Antibody Mediated Cytotoxicity.
Koestler WJ, Brodowicz T, Tomek S, Hejna M, Wiltschke C, Zielinski CC. University Hospital, Vienna, Austria.

470 Expression of HER2 and HER1 (EGFR) in the Myocardium as a Cause for the Cardiotoxicity of Trastuzumab (Herceptin).
Landt SD, Fuchs I, Evers K, Buehler H, Kühl W, Schaller G. University Hospital Benjamin Franklin, Berlin, Germany; University Hospital Charite, Berlin, Germany.

471 Defective Expression of Adhesion Molecule CD54, CD80 and CD86 Resulting in a Defective Antigen-Induced T-Cell Proliferation in Breast Cancer.
Wolfram RM, Budinsky AC, Kubista M, Kubista E, Zielinski CC. Clinical Division of Oncology, Vienna, Austria; Chair of Medical Experimental Oncology, Ludwig Boltzmann Institute for Clinical Experimental Oncology, Vienna, Austria; Vienna, Austria; Clinical Division of Special Gynaecology, Austria.

Tumor Biology

472 First Identification of Tumor Specific Antigen Mage-b3 in Two Transgenic Breast Tumor Models Driven by c-myc and v-Ha-ras Genes.
Sypniewska R, Bearss D, Windle J, Gravecamp C. Institute for Drug Development, San Antonio, TX; Arizona Cancer, Tucson, AZ; VCU, Richmond, VA.

473 Tumor/Bone Marrow Dynamic Reciprocity: Intimate Interactions with Endothelium May Confer Selective Advantage to Breast Cancer Cells.
Nunes RA, Veiga JP, Barata J, Nadler LM, Cardoso AA. Dana-Farber Cancer Institute, Boston, MA.

474 ATP and Metastatic Breast Cancer: Prognostic and Therapeutic Implications?
Kaufman PA, Salikhova A, Sterling KM, Demidenko E, Abraham EH. Norris Cotton Cancer Center and Dartmouth - Hitchcock Medical Center, Lebanon, NH.

475 Epithelial and Stromal Clonality of Fibroadenomas and Phyllodes Tumors of the Breast.
Kuijper A, Buerger H, Simon R, Schafer KL, Boecker W, van der Wall E, van Diest PJ. Free University Hospital, Amsterdam, The Netherlands; Westfalische Wilhelms University, Münster, Germany; University of Basel, Basel, Switzerland.

476 Localization of uPA-PAI1 and uPA-PAI2 Complexes in Early Breast Cancer. Correlation with Other Molecular and Biological Parameters.
Schneider J, Lucas R, Sanchez J, Tejerina A, Ruibal A. Fundacion Tejerina-Centro de Patologia de la Mama, Madrid, Spain; Universidad del Pais Vasco, Bilbao, Spain; Universidad de Alcala de Henares, Madrid, Spain; Fundacion Jimenez Diaz, Madrid, Spain.

477 **The Antiproliferative Effect of $1,25(OH)_2D_3$ on HC11 Mammary Cells is Not Associated to Induction of TGFβ and $p21^{WAF1/CIP1}$ or Inhibition of *c-myc* Expression.**
Folgueira MAAK, Katayama MLH, Snitcovsky IML, Brentani MM. Faculdade de Medicina da USP, Sao Paulo, SP, Brazil.

478 **Heparan Sulfate Proteoglycan Expression in Breast Carcinomas and Its Impact on FGF-2 Signaling.**
Mundhenke C, Maass N, Meyer K, Drew S, Friedl A. University of Wisconsin, Madison, WI; Christian Albrechts University Kiel, Kiel, Germany.

479 **The Growth Rate of Self-Detected Breast Cancers Follows a Similar (Power) Law to Mammography-Detected Cancers.**
Shochat E, Cameron DA. Western General Hospital, Edinburgh, United Kingdom.

480 **Implantation and Treatment of a Chemoresistant Breast Cancer Cell Line in a Mouse Model.**
Frühauf JH, Volz-Köster SR, Schmidt TJ, Förster C, Schneider J, Volz JO. University Hospital Mannheim, Mannheim, Germany.

SATURDAY, DECEMBER 9

7:30-9:30 POSTER SESSION V & CONTINENTAL BREAKFAST

Detection/Diagnosis

Mammography/Imaging

501 **MRI Imaging Can Predict Breast Conservation in Patients Undergoing Neoadjuvant Chemotherapy.**
Esserman L, Kaplan E, Sudilovsky D, Miller J, Hylton N. University of California-San Francisco, San Francisco, CA.

502 **Image Detected Lobular Neoplasia of Breast: Morphologic Correlation with Imaging Lesions.**
Tarjan G, Wiley EL, Spilde J, Badve S, Venta LA. Northwestern University Medical School, Chicago, IL.

503 **Mammographic Patterns and Breast Cancer Risk.**
Goyal S, Skone J, Khonji N, Clarke D, West R, Mansel RE. University of Wales College of Medicine, Cardiff, Wales, United Kingdom.

504 **Diffusion-Weighted MRI for Monitoring Treatment Response in Breast Tumors.**
Partridge SC, Esserman LJ, Tripathy D, Hylton NM. University of California, San Francisco, CA.

505 **Value of a Negative Scintimammography in the Evaluation of Patients with Abnormal Mammograms.**
Rao H, Amiruddin Q, Adaniel T, Masterson M, Archimandritis C, Likki S. Coney Island Hospital, Brooklyn, NY.

506 **The Role of Contrast-Enhanced 3D-MRI of the Breast in Detecting Local Recurrence after Surgery, Chemotherapy and Radiotherapy: Preliminary Results**
Di Seri M, Falpo S, Tomao S, Colloca ML, Manna A, Sconocchia M, Bonginelli P, Bruni A, Carrozza C, Lacava V, Potente G. Policl. Umberto I, Roma; National Cancer Institute of Genova.

507 **Mammographic Appearance of Nonpalpable Breast Cancer Predicts Pathologic Characteristics.**
Tartter PI, Gajdos C, Bleiweiss IJ, Hermann G, DeCsepel J, Estabrook A. St. Lukes-Roosevelt Hospital, New York, NY; The Mount Sinai Medical Center, New York, NY.

508 **Radionuclide Imaging of Human Breast Carcinoma Cell Line MDA-MB-231 Via In-111 DTPA-Adenosylcobalamin, In-111 DTPA-Octreotide, Ga-67 Citrate, and Tl-201 Chloride.**
Frohlich DEC, Collins DA, Hogenkamp HPC. Mayo Clinic, Rochester, MN; University of Minnesota, Minneapolis, MN.

509 **Diagnostic Value of Mammography and Breast Ultrasound for Diagnosis of Non-Palpable Lesions of the Breast.**
Ruhland FF, Heinrich JJ, Michel TT, Budner MM. Klinikum Stralsund, Stralsund, Germany.

Screening

510 **FDG-PET in Preoperative Assessment of Newly Diagnosed Breast Cancer.**
Schirrmeister HH, Kuehn T, Buck AC, Santjohanser C, Reske SN. University of Ulm, Ulm, Germany.

511 **Delay to Diagnosis Worsens Prognosis of Screen-Detected Breast Cancer.**
Olivotto IA, Gomi A, Bancej C, Brisson J, Tonita J, Kan L, Mah Z, Harrison M, Shumak R. Health Canada and the Breast Screening Programs of British Columbia, SK, AB, MB, ON, QC; Vancouver, Ottawa, Regina, Calgary, Winnepeg, Toronto, and Québec, Canada.

512 **Screening Mammography Program of British Columbia: 10-Year Outcomes.**
Olivotto IA, Kan L, D'yachkova Y, Burhenne LJW, Hayes M, Hislop TG, Worth AJ, Basco VE, King S. From the SMPBC, BC Cancer Agency and University of BC, Vancouver, Canada.

513 **The Role of Risk Factors on Multi-State Natural History of Breast Cancer: Implications for Breast Cancer Screening for Female Relatives of Breast Cancer Index Cases.**
Chen TH-H, Hsieh H-J, Yen M-F, Lai M-S. National Taiwan University, Taipei, Taiwan.

514 **Breast Health Access for Women with Disabilities: Challenges in Screening Women with Physical Disabilities.**
McKenzie SF, Cohen NR. Alta Bates Comprehensive Breast Center, Berkeley, CA.

Risk and Prevention

Familial Breast Cancer/Genetic Testing

515 **BRCA 1 / 2 Genetic Testing in Spain: A Search for Recurrent Mutations.**
Llort G, Bale AE, Blanco I, Tusquets I, Peris M, Alvarez-Franco M. Genetic Counseling Unit of Catalan Institut of Oncology, Spain; Hospital del Mar, Barcelona, Spain; Yale University, New Haven, CT.

516 **Predictors of Genetic Counseling for BRCA1/2 Among Unaffected Women**
Marcom PK, Clark S, Skinner CS, Calingaert B, Pollak KI, Sarratt WE, Sugarman J, Winer EP. Duke University Medical Center, Durham, NC; Dana Farber Cancer Institute, Boston, MA.

517 **Histologic Abnormalities in BRCA 1 and BRCA 2 Mutation Carriers Undergoing Prophylactic Mastectomies.**
Ditkoff BA, Schnabel F, Brenin D, El-Tamer M, Russo DC, Kinne D. College of Physicians & Surgeons, Columbia University, The Columbia-Presbyterian Comprehensive Breast Center, New York, NY.

518 **Descriptive Study on the Use of Prophylactic Surgery in Women with Known BRCA Mutations: The Mount Sinai Hospital Experience.**
Bordeleau L, Glendon G, Contiga V, Goodwin PJ. University Health Network, Toronto, ON, Canada.

519 **Graphical Risk Explanation as a New Method of Explaining Risk of Developing Breast Cancer to Women with a Family History - Randomised Controlled Trial to Evaluate Its Effect on Reducing Anxiety.**
Vijay V, Stein J, Saunders C, Baum M. Royal Free and University College Medical School, London, United Kingdom.

Risk Factors

520 **Breast Density: Association with Risk Factors and Stage at Diagnosis.**
Yao K, Morrow M, Hsieh Y, Rademaker F, Venta L. Northwestern University, Chicago, IL.

521 **Alteration of Stromal Protein Expression in Radial Scars of the Breast Assessed by mRNA In Situ Hybridization.**
Jacobs TW, Brown LF, Schnitt SJ. Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA.

522 **Breast Cancer Mortality and Pesticide Exposure in Brazil.**
Koifman S, Koifman RJ, Meyer A. Oswaldo Cruz Foundation, Rio de Janeiro, RJ, Brazil.

523 **Risk Factors for Breast Cancer Are Favorable Prognostic Factors.**
Tartter PI, Gajdos C, Estabrook A. St. Lukes-Roosevelt Hospital, New York, NY; The Mount Sinai Medical Center, New York, NY.

524 **Atypia on Breast Fine Needle Aspiration (FNA): Implications for Breast Cancer Risk Assessment.**
Khan SA, Yang Y, Barbescu E, Nesbitt D, Wolf A, Fontana D. Upstate Medical University, Syracuse, NY.

525 **Breast Cancer Risk Assessment: Correlation of Mammographic Patterns with Clinical Evaluation.**
Olopade OI, Huo Z, Zhong W, Nishikawa RM, White M, Rabin W, Wolverton D, Giger ME. University of Chicago, Chicago, IL.

Treatment

Chemotherapy - New Drugs and Formulations

526 **A Phase II Trial of Pemetrexed Disodium (ALIMTA™, LY231514, MTA) in Metastatic Breast Cancer Patients Who Have Failed Anthracyclines and Taxanes (Salvage Chemotherapy).**
Llombart-Cussac A, Theodoulou M, Rowland K, Lassus M, Cruciani S. Inst Valenciano de Onc, Valencia, Spain; Memorial Sloan Kettering, New York, NY; Carle Can Cntr, Urbana, IL; Eli Lilly, Indianapolis, IN; Osp. Umberto I, Lugano, Italy.

527 **Trastuzumab (Herceptin) Combined with Weekly Paclitaxel in the Treatment of Metastatic Breast Cancer: A Phase II Study.**
Scholz U, Lück HJ, Schippert C, Langer-Nitsche C, Kühnle H. University Hospital and Medical School, Hannover, Germany.

528 **1α-Hydroxyvitamin D_5 Enhances Doxorubicin-Induced DNA Breakage in ZR75-1 Breast Cancer Cells.**
Salti GI, Mehta RR, Constantinou AI, Murillo G, Das Gupta TK, Mehta RG. University of Illinois at Chicago, Chicago, IL.

529 **Capecitabine in Association with Epirubicin and Docetaxel as First Line Chemotherapy in Advanced Breast Cancer: A Dose-Finding Study.**
Angiolini C, Venturini M, Del Mastro L, Tolino G, Garrone O, Merlano M, Bergaglio M, Bertelli G, Lambiase A, Stevani I, Bighin C, Catzeddu T, Rosso R. Istituto Nazionale per la Ricerca sul Cancro, Genova, Italy; Cuneo, Italy; Milano, Italy.

530 **Treatment of HER2 Overexpressing Metastatic Breast Cancer with Trastuzumab (Herceptin®) and Chemotherapy.**
Bangemann N, Kuhle A, Willrodt RG, Buehler H, Schaller G. Free University, Berlin, Germany.

531 **A New Second Line Treatment in Advanced Breast Cancer Patients: Gemcitabine-Vindesine.**
Barni S, Ardizzoia A, Poletti P, Bernardo A, Bonciarelli G, Pancera G, Bollina R, Labianca R, Malugani F, Cazzaniga M, On the Behalf of GISCAD.U.O.. Treviglio (BG); Monza (MI); Bergamo (BG); Pavia (PV); Legnago (VR); Milano (MI), Italy.

532 **Long-Term Weekly Docetaxel in Breast Cancer - Safety Analysis.**
Breier SM, Lebedinsky CA, Ayaviri CT, Traine GO, Cot CL, Roffe CI. Hospital Israelita, Capital Federal, Buenos Aires, Argentina.

533 **The Association of Taxol and Taxotere Is Feasible and Active for Anthracycline Pretreated Metastatic Breast Cancer: Final Results of a Phase II Study.**
Gennari A, Salvadori B, Donati S, Conte P. Div of Medical Oncology - St Chiara Hospital, Pisa, Italy.

534 **Pharmacokinetics of Herceptin® Administered with Paclitaxel Every Three Weeks.**
Leyland-Jones B, Hemmings F, Arnold A, Gelmon K, Verma S, Ayoub J-P. McGill University, Montreal, Canada; Roche Products Ltd., Welwyn Garden City, Hertfordshire, United Kingdom; Hamilton Regional Cancer Centre, Hamilton, ON, Canada; BCCA Vancouver Centre, Vancouver, BC, Canada; Ottawa Regional Cancer Centre, Ottawa, ON, Canada.

535 **Efficacy and Safety Profile of Capecitabine (Xeloda®) in Combination with Paclitaxel in Patients with Locally Advanced or Metastatic Breast Cancer: Preliminary Results of a Phase II Study.**
Perez-Manga G, Batista N, Constenla M, Guillem V, Carabantes F, Ahlgren J, Castellanos J, Gonzalez-Baron M, Villman K, Söderberg K, Casinello J, Murias A, Regueiro P. H. Gregorio Marañon, Madrid, Spain; H. Montecelo, Pontevedra, Spain; I.V.O., Valencia, Spain; H. Carlos Haya, Malaga, Spain; Academic H., Uppsala, Sweden; H. Xeral Cies, Vigo, Spain; H. La Paz, Madrid, Spain; Örebro Med. Center, Örebro, Sweden; Central H., Karlstad, Sweden; H. General, Guadalajara, Spain; H. Insular, Las Palmas, Spain; Roche S.A., Madrid, Spain; H. Universitario, Tenerife, Spain.

536 **Escalating Doses of Docetaxel and Epirubicin as First Line Therapy for Metastatic Breast Cancer. A Phase I/II Study of the National Cancer Institute of Canada - Clinical Trials Group.**
Trudeau ME, Crump MR, Latreille J, Pritchard KI, Palmer M, Tu D, Shepherd L, Shear N, Shapiro L, Oldfield S, Burnell M, Vandenberg TA, Gelmon KA, Blackstein ME, Noel D. Sunnybrook & Women's College Health Sciences Centre, Toronto, ON, Canada; The Toronto Hospital - General Division, Toronto, ON, Canada; Hotel Dieu de Montreal, Montreal, QC, Canada; National Cancer Institute - Clinical Trials Group, Kingston, ON, Canada; Saint John Regional Hospital, Saint John, NB, Canada; St. Joseph's Health Centre, London, ON, Canada; BC Cancer Agency, Vancouver, BC, Canada; Mount Sinai Hospital, Toronto, ON, Canada; Aventis Pharma, Montreal, QC, Canada.

537 **Phase I Study of Weekly Docetaxel in Combination with Capecitabine in Patients with Solid Malignancies.**
Villalona-Calero MA, Shapiro C, Otterson GA, Hauger M, Kraut E, Clinton S, Shah M, Stanek M, Monk JP. Arthur James Cancer Center and R Solove Research Institute, Ohio State University, Columbus, OH.

538 **A Phase I Study of Cyclophosphamide, Doxorubicin (Adriamycin) and 5-FU/Eniluracil (CAFE) in Women with Advanced Breast Cancer.**
Bunnell CA, Parker L, Burstein HJ, Shulman LN, Scheib RG, Campos SM, Elias AD, Matulonis UA, Harris L, Younger J, Kuter I, Clarke K, Winer EP. Dana-Farber Cancer Institute and Brigham and Women's Hospital, Boston, MA; Massachusetts General Hospital, Boston, MA.

539 **Phase I Study of Vinorelbine and Capecitabine in Advanced Breast Cancer.**
Nolè F, Catania C, Mandalà M, Zampino MG, Munzone E, Ferretti G, Curigliano G, Marrocco E, Lambiase A, Goldhirsch A. European Institute of Oncology, Milan, Italy; Roche, Italy.

540 **Long-Term Follow-Up Results of Metastatic Breast Cancer Patients Treated with S-1 (a New Generation of UFT).**
Saeki T, Takashima S, Horikoshi N, Sano M, Kimura M, Miura S, Morimoto K, Noguchi S, Taguchi T, the Breast Working Group, and the S-1 Cooperative Study Group. Tokyo, Japan.

541 **Inositol Hexaphosphate Enhances Growth Inhibition of Breast Cancer Cells by Tamoxifen and Adriamycin.**
Tantivejkul K, Vucenik I, Eiseman J, Shamsuddin A. University of Maryland School of Medicine, Baltimore, MD.

542 **Antisense Oligonucleotide Therapeutics as a New Approach to Breast Cancer Therapy: *In Vitro* and *In Vivo* Studies.**
Wang H, Zhang R. University of Alabama, Birmingham, AL.

543 **Phase I-II Trial in Locally Advanced Breast Cancer with Increased Dose of Continuous Orzel (UFT + Folinic Acid) in Combination with Doxorubicin and Paclitaxel Every Three Weeks.**
Zorrilla M, Martinez-Trufero J, Puertolas T, Corral M, Artal A, Herrero A, Alonso V, Anton-Torres A. Hospital Miguel Servet, Zaragoza, Spain.

544 **A Phase II Pharmacokinetic and Pharmacodynamic Trial of 9-Nitrocamptothecin in Patients with Metastatic Breast Cancer.**
Miller KD, Haney LG, Guiney P, Murry DJ, Hawes JW, Lenaz L, Sun S-L, Sledge GW. Indiana University, Indianapolis, IN; Purdue University, West Lafayette, IN; SuperGen, Inc., San Ramon, CA.

545 **Phase I Study of Eniluracil Plus Oral 5-Fluorouracil in Combination with Docetaxel for the Treatment of Pateints with Metastatic Breast Cancer: Preliminary Results.**
Rivera E, Ricks R, Valero V, Cristofanilli M, Rosales M, Hortobagyi G. U.T.M.D. Anderson Cancer Center, Houston, TX.

Male Breast Cancer

546 **Male Breast Carcinomas Do Not Show Amplification of the HER-2/*neu* Gene.**
Bloom KJ, Reddy V, Green L, Gattuso P. Rush Presbyterian St. Luke's Medical Center, Chicago, IL; Baylor College of Medicine, Houston, TX.

547 **Patterns of Body Size in Male Breast Cancer and Impact of Obesity on Disease Outcome - The Toronto Experience.**
Madarnas Y, Franssen E, Sawka CA, Pintilie M, Goss PE. Toronto-Sunnybrook Regional Cancer Centre; Princess Margaret Hospital, Toronto, ON, Canada.

548 **Carcinoma In Situ of the Breast in Males and Subsequent Invasive Breast Cancer.**
Yap J, Chuba PJ, Aref A, Weiss L, Hamre MR. El Paso Cancer Treatment Center - Texas Oncology P.A., El Paso, TX; St. John Medical Center, Gross Pointe, MI; Barbara Ann Karmanos Cancer Institute, Wayne State University School of Medicine, Children's Hospital of Michigan, Detroit, MI.

549 **Men with Breast Cancer Have a Better Disease-Specific Survival When Compared to Women.**
El-Tamer MB, Brenin D, Andrea T, Schnabel F, Ditkoff BA, Kinne D. Columbia University, New York City, NY.

550 **Estrogen and Progesterone Receptors Comparison in Age-Matched Men and Women with Breast Cancer.**
El-Tamer MB, Hibshoosh H, Troxel A, Brenin D, Schnabel F, Ditkoff BA. Columbia University, New York, NY.

551 **Immunohistochemical Characterization of Mammary Carcinomas in Men.**
Gatalica Z, Tibbs RF, Lele SM, Mosunjac M, Palazzo JP. The University of Texas Medical Branch, Galveston, TX; Emory University, Atlanta, GA; Jefferson Medical College, Philadelphia, PA.

Psychosocial Aspects

552 **Structured Exercise Improves Physical Functioning in Women with Breast Cancer: Results of a Randomized Controlled Trial.**
Segal R, Evans WK, Gayton J, Woodard S, Wells G, Reid R. University of Ottawa, Ottawa, ON.

553 **Quality of Life in the Anglo-Celtic Randomised Trial of High Dose Adjuvant Chemotherapy.**
Forbes AJ, Foster E, Lind MJ, Twelves C, Wilson CB, Crown JP, Leonard RCF, on Behalf of the Anglo Celtic Co-Operative Oncology Group and the Scottish Cancer Therapy Network. Western General Hospital, Edinburgh, United Kingdom; Scottish Cancer Therapy Network, Edinburgh, United Kingdom; Princess Royal Hospital, Hull, United Kingdom; Beatson Oncology Centre, Glasgow, United Kingdom; Addenbrooke's Hospital, Cambridge, United Kingdom; St. Vincent's Hospital, Dublin, Ireland.

554 **An Informatics System Designed to Assist in Making Breast Cancer Adjuvant Therapy Decisions.**
Ravdin PM, Siminoff LA, Davis GJ, Parker HL, Hewlett J. University of Texas Health Science Center, San Antonio, TX; Case Western Reserve University, Cleveland, OH.

555 **Results of a Randomized Trial of a Computerized Decision Aid "Adjuvant!" to Present Tailored Prognostic Information to Stage I-III Breast Cancer Patients.**
Siminoff LA, Ravdin PM, Peele P, Silverman P, Mercer MB, Hewlett J, De Los Santos L, Parker HL, Gordon N. Case Western Reserve University, Cleveland, OH; University of Texas Health Science Center, San Antonio, TX; University of Pittsburgh, Pittsburgh, PA.

556 **Surgeons as Counsellors.**
Goyal S, Bennet P, Sweetland HM, Webster DJT, Mansel RE. University of Wales College of Medicine, Cardiff, Wales, United Kingdom.

557 **The Effects of a Group Exercise Intervention for Women with Breast Cancer Currently Undergoing Chemotherapy Treatment.**
Schneider KL, Kolden GG, Strauman TJ, Woods TE, Stewart JA, Kalin NH, Ward A, Kuta J, Sanborn L, Burt K, Mullen BA. University of Wisconsin, Madison, WI; University of Wisconsin Hospital and Clinics, Madison, WI; University of Wisconsin Comprehensive Cancer Center, Madison, WI.

558 **Improving Adherence to Breast Cancer Treatment in Public Hospital Patients Presenting with Advanced Disease.**
Marcus E, Holden C, Coon J, Lubin BJ, Preisler H, Gupta-Burt S. Cook County Hospital, Chicago, IL; Rush University, Chicago, IL.

559 **Surgery Strategies, Quality of Life, and Conspicuous Psychosocial Constellations in Women with Breast Cancer.**
Dahlbender RW, Maiterth C, Meder G, Klaus W, Kreienberg R, Kuehn T. University of Ulm, Ulm, Germany.

560 **Do Women with Breast Cancer Have Differing Requirements from Hormonal Therapy by Tablet or Injection?**
Fellowes DL, Fallowfield LJ, Houghton J, Saunders CM. Royal Free and University College Medical School, London, United Kingdom.

Tumor Cell Biology

Angiogenesis

561 **Is Angiogenesis a Predictor of the Development of Bone Metastasis? Results of Immunohistochemical Study Using CD31 and Factor VIII for Angiogenesis Assessment in Primary Breast Cancer.**
Gehani SA, Parbhoo SP, Hatter T, McDermott N, Levine T. Royal Free Hospital, London, United Kingdom.

562 **Angiogenesis in Ductal Carcinoma In Situ (DCIS) of the Breast.**
Teo NB, Shoker BS, Jarvis MC, Holcombe C, Martin L, Sloane JP. University of Liverpool, United Kingdom; Royal Liverpool University Hospital, Liverpool, United Kingdom.

563 **Microvessel Density and Vascular Endothelial Growth Factor (VEGF) Expression in Infiltrating Lobular Mammary Carcinoma.**
Chhieng DC, Marley EF, Tabbara SO, Talley LI, Frost AR. University of Alabama at Birmingham, Birmingham, AL; Washington University School of Medicine, St Louis, MO; George Washington University Medical Center, Washington, DC.

564 **Vascular Endothelial Growth Factor: A Surrogate Marker of Response in Neoadjuvant Chemotherapy of Breast Cancer? Preliminary Results of a Clinical Trial.**
Ernhardt B, Blohmer JU, Schuerenkaemper P, Lichtenegger W. Charité, Berlin, Germany.

565 **Higher Cytosolic VEGF Content from Ductal Carcinoma In Situ (DCIS) Specimens of the Breast, Than in Benign Adenomas or Invasive Breast Carcinomas: Predicting Increased Risk of Local Relapses?**
Linderholm B, Sjödin M, Tavelin B, Grankvist K, Henriksson R. Umeå University Hospital, Sweden.

566 **Vascular Endothelial Growth Factor: Cytosol Levels in Primary Breast Cancer and Correlation to Established Prognostic Factors.**
Hagen DB, Bauerfeind I, Konecny G, Kahlert S, Nestle-Kraemling C, Boettcher B, Untch M. Ludwig- Maximilians-Universitaet Muenchen, Muenchen, Germany.

567 **Comparative Assessment of Lymphagenesis and Angiogenesis in Breast Carcinomas and Measurement of the Expression of Vascular Endothelial Growth Factor (VEGF-C, VEGF-A).**
Jacquemier JJD, Mathoulin-Portier MPMP, Charafe-Jauffret EE, Viens PP, Birnbaum DD. Institut Paoli Calmettes, Marseille, France.

568 **Prognostic Significance of Angiogenesis Associated with Long-Term Survival in 377 Japanese Patients with Breast Cancer.**
Kato T, Kameoka S, Kimura T, Tanaka S, Nishikawa T, Kasajima T. Tokyo Women's Medical University, Tokyo, Japan.

569 **A Combination of NK4, a HGF/SF Variant and Gamma Linoleic Acid Increases Inhibition of HGF/SF Stimulated Human Vascular Endothelial Cell Angiogenesis.**
Martin TA, Matsumoto K, Nakamura T, Mansel RE, Jiang WG, and the Metastasis Research Group. University of Wales College of Medicine, Cardiff, Wales, United Kingdom; Biomedical Research Centre, Osaka University Medical School, Osaka, Japan.

Cell Biology

570 **Serum Deprivation Activates the Na+/H+ Exchanger and Invasion Via PKA-Dependent Phosphorylation of RhoA and Subsequent Down-Regulation of ROCK and p38 MAPK.**
Reshkin SJ, Bellizzi A, Cardone R, Paradiso A, Tommasino M, Casavola V. Univ. of Bari; Oncology Institute of Bari, Italy; Deutsches Krebsforschungszentrum, Heidelberg, Germany.

571 **Interaction of the Differentially Expressed S100A7 Gene with Centrosomal Proteins.**
Emberley ED, Hole AK, Gietz RD, Murphy LC, Watson PH. University of Manitoba, Winnipeg, MB, Canada.

572 **The RXR-Selective Retinoid LGD1069 Inhibits Breast Cell Growth through Cell Cycle Blockade and Activation of Other Receptor Pathways.**
Wu K, Tin-U CK, Yang DJ, Lamph WW, Brown PH. Baylor College of Medicine, Houston, TX; Ligand Pharmaceuticals, Inc., San Diego, CA.

Drug Resistance

573 **Antitumor Action of Estradiol on Estrogen-Deprived or Raloxifene-Resistant Human Breast Cancer Cells.**
Liu H, Lee E-S, De Los Reyes A, Jordan VC. Northwestern University Medical School, Chicago, IL.

574 **The Role of Differential Expression of Extracellular Matrix Proteins Regarding Chemoresistance of Breast Cancer Tissue in Nude Mice.**
Förster CEC, Volz-Köster SR, Schneider J, Volz JO. University Hospital Mannheim, Mannheim, Germany.

575 **Decreased Response to Paclitaxel Versus Docetaxel in a HER-2/neu Transfected Cell Line.**
Witters LM, Santala SM, Leitzel KE, Lipton A. Penn State College of Medicine, Hershey, PA.

Molecular Biology

576 **Molecular Classification of High Risk Breast Cancer Patients by Gene Expression Profiling.**
Ahr A, Karn T, Strebhardt K, Holtrich U, Kaufmann M. Universitäts-Frauenklinik, Frankfurt, Germany.

577 **Microarray Analyses of Gene Expression Regulated by ErbB-2: Interactions with Estrogen Receptor Activation/ Inhibition.**
Yang XH, Liu X, Benz CC, Thor AD. Northwestern University/ENHRI, Evanston, IL; University of California, San Francisco, CA.

578 **Prospective Study of Signal Transduction Pathways Associated with Response and Resistance to Herceptin®-Based Therapy for Patients with Metastatic Breast Cancer.**
Bacus SS, Hortobagyi G, Esteva FJ. Quantitative Diagnostics Laboratory, Elmhurst, IL; UTMD Anderson Cancer Center, Houston, TX.

579 **Somatic Genetic Alterations in Inflammatory Breast Cancer.**
Lerebours F, Bièche I, Bertheau P, Turpin E, Plassat F, Vidaud M, De Roquancourt A, Janin A, De Thé H, Lidereau R. Centre René Huguenin, Saint-Cloud; Hôpital Saint-Louis, Paris; Paris V, France.

580 **A Rapid and Cost-Effective Method for Detecting Gene Mutations in Breast Carcinomas: Screening for TP53 Sequence Variants Using Denaturing High Performance Liquid Chromatography (DHPLC) on the WAVE™ Platform.**
Lilleberg SL, Devaney JM, Lamb K, Robinson M. Transgenomic, Inc., Omaha, NE.

9:30-10:00 PLENARY LECTURE V

Digital Mammography
Laurie L. Fajardo, MD
Johns Hopkins Outpatient Center
Baltimore, Maryland

10:00-12:30 MINI-SYMPOSIUM III

Novel Targets for the Treatment of Breast Cancer
Powel H. Brown, MD PhD, Moderator
Baylor College of Medicine, Houston, Texas

10:00 **Introduction**

10:00 **DNA Repair Pathways**
Jan HJ Hoeijmakers, PhD
Erasmus University
Rotterdam, The Netherlands

10:30 **Protein Kinase Signaling Pathways**
James R. Woodgett, PhD
Ontario Cancer Institute
Toronto, Ontario, Canada

11:00 **Targeting ErbB2 and EGFR Pathways**
Rakesh Kumar, PhD
MD Anderson Cancer Center
Houston, Texas

11:30 **Clinical Trials Using Signal Transduction Inhibitors**
Eric K. Rowinsky, MD
Cancer Therapy & Research Center
San Antonio, Texas

12:00 **Novel Agents for the Treatment of Breast Cancer**
Jose Baselga, MD
Hospital General Vall d'Hebron
Barcelona, Spain

Supported by an educational grant from Aventis Oncology

12:30 ADJOURNMENT, 23rd Annual San Antonio Breast Cancer Symposium

1:00-4:30 SATELLITE SYMPOSIUM

Targeted Therapies for the Treatment of Breast Cancer
Sponsored by Genentech BioOncology
For information, contact:
Lana Manning
Phone 972-929-1900
FAX 972-929-1901
Email: theCBCE.com

1 Early Results of Breast Cancer Lymphatic Mapping from the H. Lee Moffitt Cancer Center: No Axillary Recurrences in Breast Cancer Patients after a Negative Sentinel Lymph Node Biopsy.

Dessureault S, Dupont E, Shons A, Berman C, Ku NN, Cox C, Reintgen D. H. Lee Moffitt Cancer Center and Research Institute at the University of South Florida, Tampa, FL.

While complete axillary lymph node dissection (CALND) remains the standard of care for the management of invasive breast cancer, lymphatic mapping and sentinel lymph node (SLN) biopsy promises to be a successful and accurate alternative with significant cost savings and reduction in operative morbidity. We present here an update on the lymphatic mapping experience at the H. Lee Moffitt Cancer Center and Research Institute. 1,356 consecutive patients with suspected node-negative breast cancer were mapped between April 1994 and April 1999. Lymphatic mapping was performed using Tc99m-labelled sulfur colloid and isosulfan blue dye. A SLN was defined as any blue node and/or any hot node with ex vivo radioactivity counts greater than 10 times an excised non-SLN or in situ radioactivity count greater than 3 times the background counts. Lymphatic mapping was successful in identifying the SLN in 1,302 patients (96.0%). 373 patients (28.6%) were found to have metastatic disease either by hematoxylin and eosin stains or by cytokeratin immunohistochemistry. 929 patients (71.4%) had negative sentinel lymph node biopsies. The first 120 patients in this group all underwent CALND: One patient was found to have metastatic disease in the nodal basin (False Negative Rate = 1/120 = 0.83%). The subsequent 809 patients have been observed and followed without further nodal dissection. All patients treated with breast conservation had radiation therapy to the breast. Most patients received adjuvant chemotherapy. We have not seen any recurrences to date (mean follow-up = 20 months). Previous studies have reported 10-year nodal failure rates of 17 - 28% in patients treated without axillary dissection or radiation. One study reported a median time to axillary failure of 17.2 months, with 70% of axillary recurrences occurring by 3 years. We conclude, therefore, that SLN biopsy is a sensitive alternative to CALND for the detection of nodal metastases in patients with invasive breast cancer, and can be used to select patients who do not need CALND for local control.

2 Axillary Node Examination Is a Weaker Indicator of Metastatic Potential for Inner Quadrant Compared to Outer Quadrant Breast Cancers.

Tan WW, Herman TS, Ravdin PM. University of Texas Health Sciences Center, San Antonio, TX.

We used cases from 1987 to 1996 in the SEER database to examine whether the quadrant (Q) of tumor location within the breast affected patient prognosis. Such a demonstration might be an indication of systematic understaging of tumors less likely to have lymphatic drainage solely to the axilla. This analysis was done on cases with 0-3 positive axillary nodes (a subset for which prognostic factors other than lymph node involvement can substantially affect adjuvant choice). Patients were selected who had had at least 6 axillary nodes examined (to insure that thorough axillary staging) and for whom tumor size and location were available. The quadrant of the breast was recorded as either upper outer quadrant (UOQ), upper inner quadrant (UIQ), lower inner quadrant (LIQ) or lower outer quadrant (LOQ). All patients were <50 years of age (a subset for whom mortality is not affected in a major way by non-breast cancer related events). A multivariate analysis was done using nodal status, tumor size, lower vs upper quadrants, and inner vs outer quadrants as predictors of overall survival. There were 9,118 cases included in this multivariate analysis.

Criteria		Distribution	RR	P value
Nodes	0 vs 1-3	72% N=0	2.21	< 0.0001
Tumor Size	T1 vs T2	65% T1	2.36	< 0.0001
Quadrant	Inner vs Outer	26% Inner	0.60	< 0.0001
Quadrant	Upper vs Lower	20% Lower	—	ns

Whether the patient received any radiation therapy was an independent predictor of outcome (improving mortality with a relative risk of 0.82, p value 0.01), but this did not diminish the independent predictive important of Q (inner vs outer). Inner quadrant location was a strong predictor of decreased survival and in both patient subsets who did and who did not receive radiation therapy. The prognostic implications of inner vs outer quadrants were seen in both the node negative and node positive subsets.

We conclude that after controlling for tumor stage and local therapy that women treated for breast cancer experience decreased survival if they have inner quadrant tumors. This may be due to axillary staging being a less accurate indicator of metastatic potential in patients with inner quadrant tumors. This result has implications for the method used for sentinel node biopsies and the general prognostic assessment of patients.

3 Identification of Occult Metastatic Cells in Bone Marrow Predicts Poor Prognosis Better Than HER2/neu Status and Angiogenesis in Breast Carcinomas.

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HER2/neu gene amplification and protein overexpression as well as angiogenesis are prominent biological factors in tumor progression. Recent studies have supported their potential clinical role to stratify patients for adjuvant therapy. In order to evaluate their association with the finding of clinically occult tumor cell dissemination to bone marrow (mAb A45-B/B3) of 323 newly diagnosed patients, we analyzed HER2/neu overexpression (mAb CB11; n = 208), and CD31 expression (mAb JC/70A; n = 210) of the corresponding primary tumors, applying an automated cellular imaging system (ACIS™; Chromavision Inc.) and Chalkley counting, respectively. Bone marrow micrometastases were detected in 97 (30%) of 323 patients. At 4 years of follow up, univariate and multivariate statistics of this novel cohort of patients confirmed our previous finding (NEJM 2000; 342: 525-533) that occult metastatic cells in bone marrow are an independent prognostic factor of cancer specific survival. At this time, neither HER2/neu overexpression (score 2+ and 3+) which was found in 56 (27%) of 208 cases, nor angiogenesis (Chalkley counts of 5 microvessels) in 71 (34%) of 210 cases gave significant information on patients' prognosis. In a subgroup of 94 patients, with data available for both HER2/neu and angiogenesis, a significant correlation was found between HER2/neu overexpression (2+/3+) and high microvessel counts (5) (P<.001; paired t-test). From this interim report of an ongoing study, we conclude that the direct identification of metastatic precursor cells has the potential to improve the current stratification of patients at high risk of relapse, rather than extrapolating the disseminative capacity of an individual tumor from HER2/neu overexpression and angiogenesis.

4 Selection of High Risk Lymph Node Negative Breast Cancer Patients for Chemotherapy Can Best Be Done with the Mitotic Activity Index (MAI).

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The Multicenter Mammary Carcinoma Project (MMCP) is a prospective evaluation of the prognostic value of different features in 3,479 early invasive breast cancer patients. We here report the 8 year follow-up prognostic evaluation of 586 premenopausal lymph node-negative (LN-)patients. None of these received any form of systemic adjuvant therapy and 85 (= 14.5%) of the patients died of metastatic disease.

Univariate survival analysis showed that the following variables had prognostic significance: DNA ploidy (Mantel-Cox value = MC= 2.9, Hazards Ratio = HR= 1.6), progesterone receptor (MC= 3.0, HR= 1.6), tumor diameter (MC= 7.6, HR= 1.9), estrogen receptor (MC= 10.7, HR= 2.4), nuclear grade (MC= 18.5; HR= 8.3), histologic grade (MC= 29.6, HR= 7.3). The best prognosticator was however the Mitotic Activity Index (= MAI; MC= 55.7, HR= 5.5). The analysis of the three different constituents of histologic grade showed that tubule formation and nuclear atypicality had no additional prognostic value to the mitotic impression. However the mitotic impression had lower prognostic value (MC=33.4, HR=4.1) than the MAI.

In agreement with previous smaller studies, the MAI thus had the strongest prognostic value. Only 19 (6.1%) of 310 patients with a low (< 10) MAI died of disease, contrasting to 66 (28.3%) of 233 patients with a high (>=10) MAI. In multivariate survival analysis, only the MAI and tumor size were selected, but the prognostic contribution of tumor size was low. In all subgroups the survival of patients with MAI<10 (in an area of 1.6 square millimeters) was comparably high, likewise for MAI >=10 patients, survival was comparably low. Thus, subgroup analysis of patients with different tumor diameters, ER or PR positive or negative, and different grades, showed that in each subgroup the MAI explained the prognostic value of the feature. Based on the incidences and mortality rates of different subgroups, the expected annual number of patients and deaths per year in The Netherlands could be calculated for different subgroups. The expected gain in lifes saved due to ACT is highest when high-risk patients would be selected by the MAI (>=10), while the number of over treatments is then lowest of all selection criteria available. As the MAI is widely available, easy to perform, inexpensive, prognostically optimal and well reproducible, selection of high-risk premenopausal breast cancer patients can best be done with the MAI.

5 Indicators of Lifetime Estrogen Exposure: Effect on Breast Cancer Incidence and Interaction with Raloxifene Therapy in MORE Trial Participants.

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Increased exposure to both endogenous and exogenous estrogen has been shown to be associated with elevated risk of developing breast cancer. In the Multiple Outcomes of Raloxifene Evaluation (MORE) trial, 48 months of raloxifene therapy decreased the risk of invasive breast cancer by 72% in women with postmenopausal osteoporosis compared with placebo.

Therefore, we assessed whether indicators of lifetime estrogen exposure were relevant to breast cancer incidence in the MORE trial. **Methods**: We assessed for differential effects of raloxifene (pooled 60 and 120 mg/day doses) on risk of all breast cancers through 48 months of follow-up (N=7705) according to baseline indicators of lifetime exposure to estrogen, specifically: 1) women whose femoral neck bone mineral density (BMD) was in the highest 1/3 (≥0.649 g/cm^2) vs. those in the lowest 2/3; 2) women whose baseline body mass index (BMI) was in the highest 1/3 (≥26.40 kg/m2) vs. those in the lowest 2/3; and 3) women with the highest baseline estradiol level (≥5.0 pg/ml, the detection limit of the assay) vs. those with estradiol level <5.0 pg/ml. Effects of dichotomized baseline demographics and their interactions with treatment were analyzed using a logistic regression model. **Results**: Women with estradiol levels ≥5 pmol/L had a 2.5-fold higher breast cancer risk than women with lower levels (p=0.001). We found that the women with evidence of lower lifetime estrogen exposure experienced a reduction in risk with raloxifene therapy (56%, 64%, and 55% for the lower BMD, lower BMI, and lower estradiol groups, respectively), while women in the groups corresponding to higher estrogen exposure experienced more profound reductions in risk (94%, 82%, and 77% for the higher BMD, higher BMI, and higher estradiol groups, respectively). The differential effect of raloxifene (ie, treatment-by-subgroup interaction) on the lower- vs. higher-estrogen exposure groups was statistically significant for BMD (p=0.005). **Conclusion**: We conclude that raloxifene therapy reduces the risk of breast cancer in postmenopausal osteoporotic women regardless of lifetime estrogen exposure, but that the reduction is greater in those with higher lifetime exposure to estrogen.

6 Comparison of Efficacy and Tolerability of Fulvestrant (Faslodex™) with Anastrozole (Arimidex™) in Post-Menopausal (PM) Women with Advanced Breast Cancer (ABC) – Preliminary Results.

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'Faslodex' (ICI 182,780) (FAS) is a novel, estrogen receptor downregulator. We report here a phase III clinical trial [0020], which compared FAS and 'Arimidex' (anastrozole) (ADX) in PM women with ABC who had progressed or recurred on prior endocrine treatment for early or advanced breast cancer.

An open, randomized, multi-center, parallel-group, trial was conducted to compare the efficacy and tolerability of FAS with ADX. The primary endpoint was time to progression (TTP). Secondary endpoints included objective response (OR) rates, duration of response (DOR) and tolerability. Patients were randomized to either FAS 250mg (1 x 5ml) (n=222) i.m. once monthly or ADX 1mg (n=229) orally od. Patients were recruited between June 1997 and September 1999 and followed for a median of 305 days. At the time of analysis approximately 83% of patients in each treatment arm had progressed. Median TTP was 167 days and 156 days for FAS and ADX respectively (hazard ratio 0.97; confidence limits (CL) 0.79.[1]28; p=0.78). Objective response (OR, CR+PR) rates showed a non-significant numerical advantage for FAS (20.7%) over ADX (15.7%)(odds ratio 1.38; CL 0.84.[2]29; p=0.20), with the odds of attaining OR being 38% higher for FAS treated patients. Clinical benefit rates (CR+PR+SD≥24 weeks) were 44.5% and 45.0% for FAS and ADX respectively. Median duration of response was 434 days for FAS and 425 days for ADX.

Both treatments were well tolerated with 3.2% of FAS patients and 2.2% of ADX patients withdrawn due to adverse events. For FAS and ADX, side effects included: hot flushes, 18.6% and 17%; gastrointestinal disturbances, 40.0% and 34.3%; weight gain 0.5% and 1.7%; vaginitis 0.5% and 0.9%.

In conclusion, FAS was at least as effective as ADX, with a non-significant numerical increase in OR observed. This novel agent shows good efficacy in patients resistant to tamoxifen. These data confirm that with its good tolerability FAS is an effective treatment for ABC in PM women.

7 A Double-Blind Randomized Trial Comparing the Efficacy and Tolerability of Faslodex™ (Fulvestrant) with Arimidex™ (Anastrozole) in Post-Menopausal (PM) Women with Advanced Breast Cancer (ABC).

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Introduction 'Faslodex' (ICI 182,780) (FAS) is a novel, estrogen receptor downregulator, that has previously shown good tolerability, and clinical activity in a phase II trial in tamoxifen resistant patients with metastatic breast cancer. In North America, a phase III clinical trial comparing FAS 250mg intramuscular (i.m.) injection once monthly and 'Arimidex' (anastrozole) (ADX) 1mg od in PM women with ABC who had progressed or recurred on prior endocrine treatment for early or advanced breast cancer, has recently completed recruitment. A second study of similar design, except not double-blind, has also completed recruitment.

Materials & Methods A double-blind, randomized, multicentre, parallel-group trial was conducted to compare the efficacy and tolerability of FAS with ADX. Patients were randomized to either FAS 250mg (2 x 2.5ml) i.m. once monthly plus ADX-placebo orally od or ADX 1mg orally od plus 2 x 2.5 ml FAS-placebo i.m injections. 400 patients were recruited to the trial, with treatment received not yet unblinded. The primary endpoint of the trial was time to progression (TTP), with 392 patients required to give a 90% power to detect a difference corresponding to 60 days in median TTP. Secondary endpoints included objective response (OR) rates, duration of response (DOR) and time to treatment failure (TTF) and tolerability.

Conclusion The trial has completed recruitment. Results will be available Q4 2000, and will be presented in full.

8 Femara® (Letrozole) Showed Significant Improvement in Efficacy over Tamoxifen as First-Line Treatment in Postmenopausal Women with Advanced Breast Cancer.

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In this large multicenter, double blind, phase III study, 907 postmenopausal women with locally advanced or metastatic breast cancer were randomly assigned either letrozole 2.5 mg o.d (453 patients) or tamoxifen 20 mg o.d. (454 patients). This study was conducted in 29 countries involving 201 centers. Enrollment was over a period of 2 years. The primary endpoint was time to progression (TTP). Secondary endpoints included objective response (OR: CR and PR) and clinical benefit rates. Analysis was performed after 662 events occurred. Median follow up time was 18 months. Demographic characteristics were well balanced between treatment groups. Less than 20% of the patients had prior adjuvant anti-estrogen treatment, and approximately 10% received one course of chemotherapy for advanced disease. Patients had positive ER and/or PgR status (65%), unknown receptor status (34%) or negative status (<1%). Dominant site of disease was soft tissue for 25% of the patients, bone for 30% and visceral for 44%. Superiority for letrozole was shown in TTP (median, 41 weeks vs 26 weeks, P=0.0001, Cox regression), OR (30% vs 20%, P=0.001, logistic regression) and clinical benefit (49% vs 38%; P=0.001, Mantel-Haenszel). These results were consistently reproduced by dominant site. Median TTP in soft tissue was 56 weeks for letrozole, 28 weeks for tamoxifen; in bone 42 weeks for letrozole, 27 weeks for tamoxifen; in visceral 36 weeks for letrozole, 20 weeks for tamoxifen, (P=0.0001, logrank test). Similar superiority in OR was seen by dominant site favoring letrozole (P=0.001 Cochran-Mantel-Haenszel). Both letrozole and tamoxifen were well tolerated.

These data show the overall consistent superiority of letrozole over tamoxifen including by site of dominant disease and strongly support the use of letrozole as the first choice treatment for postmenopausal women with advanced breast cancer.

9 Sulfotransferase Expression in Normal and Cancerous Human Breast Tissues.

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The metabolism of β-estradiol (E2) in human breast tissues is important in regulating growth and development. Sulfate conjugation is a major biotransformation reaction for steroids. The sulfation of E2 prevents its binding to the estrogen receptor and increases its secretion from cells. Two human sulfotransferases (STs) have a role in E2 sulfation in breast tissues. Estrogen ST (EST) sulfates E2 at low nanomolar concentrations whereas phenol-sulfating phenol ST-1 (PPST-1) sulfates E2 only at micromolar concentrations; monamine-sulfating phenol-sulfating ST (MPST) is also present in breast tissues but does not sulfate E2. Because of its high affinity for E2 sulfation, the expression of EST in breast cancer cells greatly decreases their responsiveness to E2 stimulated growth (Oncol. Res. 9:589, 1998). EST is detected in primary human breast epithelial cells but not in human breast cancer cell lines indicating that the cancer cells lack an important mechanism for regulating the activity of E2. The PSTs are expressed only in breast cancer cell lines. To investigate the expression of EST and the PSTs in normal and cancerous breast tissue, expression of EST, PPST-1 and MPST was examined in frozen pathological samples of normal and cancerous human breast tissue using RT-PCR. EST was expressed preferentially in pre-menopausal normal breast tissue. Neither EST or the PSTs were detected in normal post-menopausal tissue samples. In contrast, EST was expressed in 30% and the PSTs in 70% of the cancerous breast tissues. To obtain pure populations of normal epithelial cells or of cancer cells Laser Capture Microdissection of frozen sections from pathological breast samples was performed. RNA from the microdissected cells was analyzed by RT-PCR for PPST-1, MPST, and EST expression. PPST-1 and MPST messages were detectable in breast carcinomas but not in normal breast epithelium. EST was not detected in breast cancer cells, indicating that its detection in frozen tumor samples may be due to contamination with normal tissue. The loss of EST in normal breast cells may result in an inappropriate estrogenic growth stimulation. This study confirms that human STs are differentially expressed in normal breast epithelium and in breast carcinoma tissue and yields valuable information as to the roles of the individual STs in estrogen metabolism in normal and breast cancer cells. (Supported by NIH grant GM38953)

10 Resistance of Mutant BRCA1 Breast Cancer Cells to Paclitaxel-Induced Apoptosis Mediated by Bcl-2.

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BRCA1 encodes a protein implicated in the response to DNA damage through activation of several protein pathways and cells with *BRCA1* mutations are hypersensitive to DNA damaging agents. Another class of chemotherapy agents active in breast cancer includes taxanes that act on microtubules and is regulated by phosphorylation and inactivation of the Bcl-2 protein.

The HCC1937 breast cancer cell line containing homozygous *BRCA1* mutations was transfected with wild-type *BRCA1* expression vectors. Parental *BRCA1* mutant HCC1937 breast cancer cells were hypersensitive to ionizing radiation, cisplatinum, and adriamycin with resulting decreased cellular survival determined by clonogenic assays compared to isogenic cells expressing wild-type *BRCA1*. However, parental HCC1937 *BRCA1* mutant cells treated with 20 nM paclitaxel demonstrated a 3-5-fold increase in clonogenic survival compared to the isogenic matched HCC1937 *BRCA1* expressing cells. Cellular proliferation studies revealed a 20% increase in DNA synthesis following paclitaxel treatment in *BRCA1* mutant cells compared to a 70% reduction in proliferation following paclitaxel treatment in isogenic *BRCA1* wild-type cells. We found the levels of apoptosis 48 hrs. following treatment with 20 nM paclitaxel were 22%±2% in *BRCA1* wild-type cells compared to only 5%±1% in *BRCA1* mutant breast cancer cells using both DAPI and DNA comet assay. Western blotting revealed low levels of Bcl-2 protein and lack of Bcl-2 phosphorylation in parental *BRCA1* mutant HCC1937 cells in response to paclitaxel. Transfection of wild-type Bcl-2, but not site-directed serine mutated Bcl-2, into parental HCC1937 cells resulted in restoration of normal paclitaxel-induced apoptosis. Immunohistochemical staining of *BRCA1* mutant breast tumors revealed 2/21 (10%) demonstrated expression of Bcl-2 compared to Bcl-2 immunoreactivity in 68/100 (68%) of sporadic breast tumors ($p<0.01$).

BRCA1 mutant breast tumors have low levels of Bcl-2 protein which resulted in resistance to paclitaxel-induced apoptosis. These findings may have important treatment implications for breast cancer patients with inherited *BRCA1* mutations and suggest that treatment with paclitaxel may not result in optimal response.

11 Akt2 Upregulation in HER-2/neu Overexpressing Breast Cancers: Implications to Their Clinical and Biological Behavior.

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The ability of trophic factors to promote survival has been attributed at least in part, to the phosphatidylinositide 3'-OH kinase (PI3K)/C-Akt kinase cascade. Several targets of the PI3K/c-Akt signaling pathway have recently been identified. For example, PTEN has also been identified as a human tumor suppressor whose loss correlates with increased Akt activity and has significant implication for current models of oncogenesis and drug resistance. In some circumstances, Akt can induce cell cycle progression. Akt can suppress apoptosis through deletion of PTEN, the overexpression of active Ras, or the overexpression of active PI3K, which suggests that oncogenes may block cellular apoptosis by hyperactivating Akt. Recent studies have demonstrated that Akt regulates apoptosis at multiple sites and has identified direct Akt targets including Bad, caspase 9, the Forkhead family of transcription factors and the NFkB regulator IKK. Specifically, Akt2 plays an important role in cell survival and in blocking programmed cell death or apoptosis after radiation or chemotherapy. Akt1 and Akt2 have been shown to be activated in cells overexpressing HER-2/neu.

The association of HER-2/neu overexpression with the activation of the PI3 kinase pathway prompted us to study the level of expression of Akt proteins in breast cancer cell lines MCF7 transfected with HER-2/neu (MCF7/HER-2) using the microarray technique. We found that both Akt1 and Akt2 as well as their downstream target, NF6B, were upregulated. These results were confirmed by northern blot analysis. To explore the importance of this finding for breast cancers, we analyzed the levels of Akt1 and Akt2 in a series of breast cancers on which the HER-2/neu status was known. We found that in patients with HER-2/neu overexpression, Akt was activated and Akt-2 was significantly upregulated. In addition, inhibition of PI3 kinase and Akt2 by specific inhibitors resulted in an increased sensitivity to cellular killing by UV radiation and hypoxia. Our results show that upregulation of Akt2 and its pathways may be implicated in drug resistance and aggressive behavior of cancers overexpressing HER-2/neu.

12 Telomerase Activity in Human Breast Cancer.

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Telomerase is a ribonucleoprotein enzyme which appears to play an important role in carcinogenesis. Telomerase reactivation seems to be associated with immortalisation and malignancy. A proposed aetiology of tumour activation involves p53 mutations.

Using a polymerase chain reaction (PCR) based assay, we examined telomerase activity in 77 breast tissue specimens including infiltrating carcinoma (n=43), adjacent non-cancerous breast (n=21), benign breast disease (n=5) and ductal carcinoma in situ (DCIS) (n=8). The expression of p53, Ki67, oestrogen receptor (ER) and progesterone receptor (PR) was determined in infiltrating carcinoma using immunohistochemistry. The histopathological features were determined by light microscopy by an experienced breast pathologist.

Telomerase activity was detected in 32 (74%) of 43 invasive breast cancers and in none of DCIS, benign or normal breast tissue specimens. There was no statistically significant association between telomerase activity, tumour grade, p53 protein accumulation and ER/PR expression. Telomerase activity was significantly associated with larger tumour size, the presence of lymphovascular invasion, nodal metastases and increased cellular proliferation. Furthermore, our experience with the immunohistochemical detection of hTERT (the catalytic subunit of telomerase) in breast cancer is also presented.

13 Zoladex™ (Goserelin) Vs. CMF as Adjuvant Therapy in Pre-/Perimenopausal Early (Node Positive) Breast Cancer: Preliminary Efficacy, QOL and BMD Results from the ZEBRA Study.

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This study was set up in 1990 to show equivalence in disease-free survival (DFS) of Zoladex with cyclophosphamide, methotrexate and 5-fluorouracil (CMF) in pre-/perimenopausal early breast cancer. 1640 patients were randomised to Zoladex 3.6 mg (2 years, n=817) or CMF (6 cycles, n=823). Quality of life (QoL) was assessed in approx. 1000 patients pretreatment, at 3 and 6 mths, and at 1, 2 and 3 yrs or until recurrence or death using the Rotterdam Symptom Checklist (RSCL). This consists of four dimensions: physical distress, psychological distress, activity levels and overall quality of life. Hormonal and social effects and efforts to cope with illness were also measured. Bone mineral density (BMD) was measured at baseline, 1, 2 and 3 yrs in selected centres.

Prognostic factors were well balanced between treatment groups. A significant interaction was observed between treatment and ER-status (p=0.0016), therefore protocolled subgroups were analysed separately.

In ER+ve patients (~72%,), Zoladex was equivalent to CMF for DFS (HR=0.99, 95%CI 0.83-1.19). In ER-ve patients (~19%), HR was in favour of CMF for DFS (HR=1.72; 95%CI 1.24-2.37).

Side effects were as expected: alopecia and nausea/vomiting for CMF; hot flushes and vaginal dryness for Zoladex. Overall QoL score, physical distress score, effort to cope with illness and activity level were significantly better for Zoladex than for CMF at 3 and 6 mths, covering the CMF treatment period. Zoladex was comparable to CMF for psychological distress score and social effects. Hormonal effects were significantly better for CMF than for Zoladex over 2 yrs, but were significantly different in favour of Zoladex at 3 yrs (after Zoladex treatment had finished).

BMD loss in both groups was observed whilst on treatment; this loss was slightly greater at 1 and 2 yrs (ie during treatment) in the Zoladex group; however, recovery of BMD was observed post-treatment with Zoladex, whereas CMF patients continued to lose BMD (preliminary data).

Zoladex alone offers an alternative to adjuvant chemotherapy in ER+ve, pre/perimenopausal early breast cancer, showing equivalent efficacy and benefits in terms of QoL without the distressing side effects associated with CMF. BMD loss was observed in both groups but post-treatment recovery was seen only in the Zoladex group.

14 A Randomized Double-Blind Multicenter Study of Pre-Operative Tamoxifen Versus Femara™ (Letrozole) for Postmenopausal Women with ER and/or PgR Postive Breast Cancer Ineligible for Breast-Conserving Surgery. Correlation of Clinical Response with Tumor Gene Expression and Proliferation.

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Preoperative tamoxifen (TAM) reduces tumor size before surgery, potentially assisting breast-conserving surgery (BCS) for postmenopausal (PMP) women with estrogen receptor (ER) positive breast cancer. Better selection of patients, together with more potent endocrine therapy, are required to increase the efficacy of this non-toxic neoadjuvant approach. To address these issues, the selective aromatase inhibitor letrozole (LET) 2.5 mg daily was compared in a double-blinded study with TAM 20 mg daily as 4 months preoperative treatment for 337 PMP patients with large (>2cm) ER and/or PgR positive breast cancer ineligible for BCS. Tumor molecular markers (MM) were analyzed before and after treatment and included: ER, progesterone receptor (PgR), Trefoil factor 1 (PS2), ErbB2, epidermal growth factor receptor (EGFR) and proliferation index (Ki67). For the 324 evaluable patients (154 LET, 170 TAM) the clinical complete and partial response rate was 55% for LET and 36% for TAM (P<0.001). 45% of LET patients underwent BCS versus 35% for TAM (P=0.022). MM analyses were available for 86% of cases pretreatment, and in 69% a post-treatment sample was also available. The two treatment groups did not differ with respect to any pretreatment MM, thus the efficacy difference between LET and TAM was not due an imbalance in baseline tumor characteristics. ErbB2 was expressed in only 14% overall and ER analysis was more predictive for response than PgR analysis. The influence of MM on response and changes in MM with treatment will be presented. In appropriately selected PMP patients with hormone-sensitive breast cancers, 4 months of preoperative LET is associated with higher rates of tumor response and BCS than TAM.

15 Distinct Deletions in the egfr Gene Enhancing Transcription Force Tumor Growth in Breast Carcinogenesis.

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The segregation of alleles associated with a high transcriptional activity of the egfr gene are first candidates to play a decisive role in breast carcinogenesis. We identified loss of heterozygosity at a polymorphic CA repeat (CA-SSR) in intron 1 of the egfr gene in 55 of 163 primary breast cancer cases. Mapping of the chromosomal region around this egfr CA-SSR using 5 additional CA-SSR markers in intron 1 and 8 CA-SSR markers in 7p11-14 showed that either a isolated deletion in egfr CA-SSR in intron 1 or the total loss of the egfr gene on one chromosome occurred. Haplotypes of the polymorphism containing a short CA-stretch remained in two third of the cases which have been proved experimentally to determine a high egfr transcription activity in vitro and in tumor tissue. In 12/16 of these patients a LOH in the CA-SSR of the egfr gene in at least two normal, non-tumorous breast lobules was detected. Therefore, we conclude that this genetic alteration is a primary event in breast carcinogenesis. To our knowledge no other genomic alteration leading to gene deregulation has so far been reported with such a high frequency in non-tumorous breast tissue. The segregation of the LOH was further detected in all cases of associated ductal carcinoma in situ (DCIS) and also in all lymph node metastases. In a first case control study the patients allele frequencies were compared to 192 Caucasian healthy controls. A higher cancer risk was associated with the inheritance of heterozygosity for a short and a long egfr CA-SSR allele (Odds ratio 10.8, p <0.001). Further we compared the data to those of Asian women who are on average under a four time lower risk of breast cancer that Caucasians from europe. 179 Asian healthy controls and 95 patients displayed a 7-8 times higher frequency of two long egfr CA-SSR alleles than the Caucasians. In the tumors the shorter allele was conserved 4 times more frequent than the longer which underlines that allele-specific gene expression due to length and LOH of the egfr CA-SSR could be assumed to be an important event in breast carcinogenesis.

16 Cytogenetic Differences Revealed by Comparative Genomic Hybridization (CGH) in Japanese and German Breast Cancer Cases.

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The incidence of invasive breast cancer differs throughout the world with the highest incidences in the western world and a four times lower incidence in Japan associated with a slightly better prognosis and a higher degree of tumour differentiation. Factors thought to contribute to these different incidences are life style habits, especially different food intakes, nevertheless epidemiological studies also point to genetic factors.

To give insights in genetic differences of breast cancer cases from german and japanese patients we investigated 41 japanese and 161 german sporadic breast cancer cases by means of Comparative Genomic Hybridization (CGH) and correlated these findings with the menopausal status and estrogen receptor expression.

Overall german breast cancer cases revealed a slightly higher number of geentic alterations per cases in contrast to japanese breast cancer cases. This was accentuated in premenopausal japanese breast cancer cases which showed a significantly lower average number of genetic alterations per case in contrast to their german counterparts (6.3 vs. 9.6; p<0.05). Both subgroups differed strikingly in the rate of losses of 17p-material with a rate of 63% in japanese patients and 21% in german patients (p<0.0001). Estrogen receptor expression was associated with a lower number of genetic alterations per case in both subgroups (7.6 vs. 10.5 in the german population; p<0.01 and 6.1 vs. 9.0 in the japanese population; p=0.11) and the loss of 16q-material (p<0.01 in both subgroups). In addition, japanese, estrogen receptor negative cases were associated with an increased rate of 8p-losses (p<0.01).

To our knowledge these data for the first time show significant genetic differences in breast cancer cases of different ethnic subgroups giving rise for the hypothesis that the different associated clinical and pathological parameters are reflected on a genetic level.

17 Detection of Pre-Malignant and Malignant Breast Cells by Ductal Lavage.

Dooley WC, Veronesi U, Elledge R, O'Shaughnessy J, Ljung B-M, et al. Johns Hopkins, Istituto Europeo di Oncologia, Baylor Houston, Baylor Dallas, UCSF, et al[1].

All ductal and lobular breast cancers originate in the single layer of epithelial cells that line the ductal/lobular units of the breast milk ducts. We performed ductal lavage (DL) using a novel microcatheter to recover ductal cells to determine the presence of pre-malignant and malignant cells in breasts of high-risk women with non-suspicious mammograms and physical exams. **Methods:** High-risk women (Gail index≥1.7, previous breast cancer or BRCA1 or BRCA2 +) were lavaged with the Pro•Duct™ Health microcatheter. Cells collected in lavage fluid were cytologically analyzed and diagnoses were rendered as either insufficient cellular material for diagnosis (ICMD), benign (B), atypical (A), either with mild (AMLD) or marked (AMKD) changes, or malignant (M). **Results:** DL was performed on 422 high-risk women and a total of 543 breasts. There were no serious adverse events related to the procedure. 449 DL specimens from patients meeting the above entry criteria were cytologically analyzed. 16.3% (73/449) and 4.7% (21/449) of lavaged breasts yielded a cytological diagnosis of AMLD and AMKD respectively, for a combined atypia prevalence of 21% (94/449). 1 of 449 lavaged breasts yielded a cytological diagnosis of frank malignancy (M). In this case, a 1.8 cm high-grade DCIS with focal comedo necrosis was confirmed and resected with clear margins following exploration and partial resection of the identified duct. In 1 of 21 cases of AMKD so far, duct exploration and resection has confirmed a 6 cm DCIS lesion in the duct yielding markedly atypical DL cytology. Pathological examination of a 3rd case has revealed multiple papillomas with atypical hyperplasia. Work-up of 2 more cases with repeat mammograms, MRI, ultrasound and ductoscopy revealed papillomas but no high-grade intraductal lesions correlating with the lavage cytology although ductoscopy is obviously limited in its ability to access and sample all ductal branches. The other 17 cases of AMKD are still undergoing work-up. **Conclusions:** Ductal lavage is a safe, well-tolerated and minimally invasive procedure for determining the presence of pre-malignant and malignant cells in the breast. Ductal lavage may be a useful adjunct to mammography and other currently available imaging modalities for the early detection of intraductal breast pathology. [1]Preminary results presented at ASCO May 2000

18 Fiberoptic Ductoscopy for Patients with Nipple Discharge.

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Background: Breast cancer and pre-cancer are thought to start in the lining of the milk duct or lobule yet we have not had, until recently, direct access to this area other than in tissue removed blindly by core biopsy or fine-needle aspiration. Fiberoptic ductoscopy is an emerging technique to allow direct visual access to the ductal system of the breast through nipple orifice exploration.

Methods: We applied ductoscopy to 382 women with nipple discharge and analyzed the visual findings, the cytological washings and the subsequent histopathology.

Results: In 174 (45.5%) of these women, fiberoptic ductoscopy was successful in visualizing an intraductal papillary lesion. Of these visualized cases, 140 (80.5%) had a single papilloma, 31 (17.8%) had multiple discrete papillomas, and 3 (1.7%) had diffuse intraductal thickening which corresponded to diffuse papillomatosis on histopathological analysis. The overall positive predictive value of FDS screening was 83%. Of the lesions observed, 37.6% were located in the main (segmental) duct, 35.3% lesions in the first branch, 21.6% lesions in the second branch, 4.5% in the third branch and 0.9% in the fourth branch with an overall average distance of 2.54 cm from the nipple orifice. Ductal washings done at the time of ductoscopy were effective at obtaining representative exfoliated ductal cells which could be evaluated for the presence of clumps (>50 cells), clumps with atypia or single ductal cells. The presence of clumps with positive FDS increased the positive predictive value to 90%.

Conclusions: Fiberoptic ductoscopy presently offers a safe alternative to ductography in guiding subsequent breast surgery in the treatment of nipple discharge.

19 FDG-PET and Lymphoscintigraphy (LS): Towards a Comprehensive Evaluation of the Internal Mammary (IM) Lymph Nodes.

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Controversy regarding the detection and management of IM lymph node metastases in patients with breast cancer continues to increase. Two recent randomized trials showed a survival advantage to regional nodal radiation after mastectomy, but the relative contribution of IM radiation remains unclear. We hypothesize that PET and LS can function in a complementary fashion, with PET diagnosing macrometastases, and LS identifying drainage patterns to IM nodes which may harbor microscopic disease. We present the results of 3 studies that support this approach. In a group of 73 patients with recurrent/metastatic disease, the prevalence of suspected disease in mediastinal or IM nodes was higher with PET (40%) than CT (23%). In the subset of patients with biopsy or CT confirmation (n=33), the sensitivity and specificity for PET was 85% and 90% respectively, and for CT 54% and 85% ($p<.05$). In the subset of patients suspected of having only locoregional disease (n=33), PET identified unsuspected IM/mediastinal disease in 10 (30%). In a more uniform set of 30 patients with locally advanced disease undergoing neoadjuvant chemotherapy, 23% were found to have highly suspicious IM uptake of FDG. All PET positive patients had negative CT imaging. Characteristics predictive of IM disease included inflammatory breast disease (4/7 patients with IBD had + IM) and palpable axillary disease (6/17 with palpable axillary disease had + IM). Interestingly, of patients with PET positive IM uptake, 3/6 had patterns of failure consistent with IM spread (pulmonary metastases), compared with 2/21 patients without IM uptake ($p=.06$). IM drainage has also been demonstrated by sentinel node mapping during axillary evaluation. Of patients undergoing LS using both technetium and blue dye, nodal drainage was documented in 184/220. 37/220 (17%) had IM node(s) identified, and 2 patients had IM only drainage. Primary breast quadrant was predictive of IM drainage: 17% upper inner, 10% upper outer, and 27% lower outer and 25% lower inner. In conclusion, the combination of PET and LS not only offers a promising approach to the detection of IM metastases, but may also provide insight into patterns of failure and allow for a more refined approach to treatment selection.

20 Prostate Epithelium-Derived Ets Transcription Factor Is a Candidate Breast Tumor Marker and a Breast Tumor Antigen.

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By using the Digital Differential Display method we have identified six cDNAs that show ~5-fold or higher representation in the cDNA libraries from human breast tumors in comparison to normal human breast tissue. In addition, these cDNAs occur at a very low or undetectable frequency in the cDNA libraries from other normal human tissues. Four of the six cDNAs encode previously known proteins including prostate epithelium-derived Ets transcription factor (PDEF), Ubiquitin binding protein p62 (UBp62), cytochrome B561 (CB561) and myosin light polypeptide 5, regulatory (MYL 5). The other two are expressed sequence tags (ESTs) identified in the database as unigenes Hs.44017 and Hs.215937 respectively. By using RT/PCR we have analyzed the expression of one of the cDNAs (PDEF) in various normal human tissues and in breast tumors and found that PDEF expression in normal human tissues is highly restricted. Thus, with the exception of normal breast tissue and trachea, PDEF was not expressed at significant levels in other normal human tissues including brain, heart, kidney, liver, lung, prostate, testis and uterus. In contrast, PDEF was over expressed in 4 out of the five primary breast tumors tested suggesting that PDEF is a potential diagnostic breast tumor marker. We plan to test 50 primary human breast tumors to get a better estimate of the frequency of over expression of PDEF in tumors. Furthermore, restricted expression of PDEF in normal human tissues and over expression in breast tumors indicates its potential as a novel breast tumor antigen. By using an online computer algorithm, the 334 amino acid residue long coding sequence of PDEF is predicted to contain many potential HLA-A2 binding peptides. We are beginning to test the immunogenicity of these peptides in HLA-A2/Kb transgenic mice. Finally, on the basis of our observations with PDEF, we suggest that one or more of the other 5 cDNAs we found to be over represented in the cDNA libraries from human breast tumors may also turn out to be novel breast tumor markers and breast tumor antigens. Our results point to a vast potential of the CGAP data base and the Digital Differential Display program in uncovering interesting molecules relevant to other human cancers that may have applications as tumor markers and tumor antigens as well as targets for cancer drug discovery.

21 Amplification of Topoisomerase II Alpha Is a Strong Predictor of Response to Epirubicin-Based Chemotherapy in HER-2/neu-Positive Metastatic Breast Cancer.

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Amplification of the HER-2/neu oncogene has been linked with altered sensitivity to cytotoxic drugs in breast cancer. We studied whether or not concurrent aberrations of the topoisomerase II alpha gene (topoIIalpha) gene, which is located close to HER-2/neu at chromosome 17, could predict response to topoisomerase II inhibitor-based chemotherapy.

Patients included in the present study (n=196) had been treated in conjuction with a previously reported randomized trial (Joensuu et al. JCO 1998;16:3720-3730), where two epirubicin-based regimens were compared as first-line chemotherapy in metastatic breast cancer. Gene copy numbers of HER-2/neu and topoII alpha were determined by fluorescence in situ hybridization. The primary end-points were clinical response to and survival after initiation of the first-line chemotherapy.

HER-2/neu amplification, present in 32% of primary tumors, was associated with both short distant disease-free and overall survival (p=0.038 and 0.0003, respectively), but not with clinical response to chemotherapy (p=0.42). TopoII alpha gene amplification, present in 34% of HER-2/neu positive tumors, defined a subgroup of patients with a significantly higher clinical response than HER-2/neu-positive patients without topoII alpha gene amplification (79% vs. 35%, p=0.0004). All patients showing a complete response (n=7) had topoII alpha gene amplification in the primary tumor. The opposite gene copy number aberration, physical deletion of topoII alpha gene, was found in 21% of HER-2/neu positive tumors. Tumors with TopoII alpha gene deletion were the least responsive to chemotherapy (response rate 17%). Patients with topoII alpha gene amplification had a similar distant disease-free survival but a significantly longer survival after initiation of chemotherapy as compared with those with no amplification (median 20 vs. 11 months, p=0.0026).

HER-2/neu alone was not a predictor of response to first-line epirubicin-based chemotherapy in metastatic breast cancer. Topoisomerase II alpha gene amplification defines a subgroup within patients with metastatic HER-2/neu-positive breast cancer who frequently respond to epirubicin-based chemotherapy.

22 Transcription Factor YB-1 Predicts Clinical Drug Resistance and Patient Prognosis in Breast Cancer.

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Development of a multi-drug resistant phenotype is primarily responsible for failure of current treatment regimens in breast cancer. Several mechanisms may be involved, including increased expression of the efflux pump P-glycoprotein. We previously reported that the Y-box factor YB-1 is involved in regulating transcription of the P-glycoprotein gene MDR1 and demonstrated that nuclear YB-1 overexpression in human breast cancer is associated with increased P-glycoprotein expression (Bargou et al, Nature Med. 3, 447-450, 1997). We now report on the clinical relevance of YB-1 expression in breast cancer (n=83) after a median follow-up of 61 months: In patients treated by postoperative chemotherapy (predominantly anthracyclin-containing regimens), the 5-year relapse rate was 66% in patients with high YB-1 expression in tumor and surrounding benign breast epithelial cells. In those patients with low YB-1, no relapse has been observed so far. YB-1 thus determines clinical drug resistance in breast cancer. Moreover, YB-1 determines breast cancer aggressiveness: In patients not treated by postoperative chemotherapy, all of those with low YB-1 are still free of disease, whereas the 5-year relapse rate in those with high YB-1 was 30%. Hence, YB-1 identifies high-risk breast cancer patients both in the presence and absence of postoperative chemotherapy, thus demonstrating strong prognostic and predictive significance. Consequently, YB-1 is a promising target molecule for development of novel therapeutic strategies aimed at overcoming multi-drug resistance and tumor aggressiveness.

23 HER2-Status Predicts Complete Pathological Response (pCR) in Primary, Operable Breast Cancer (BC) Treated with Neoadjuvant Epirubicin/Docetaxel + G-CSF.

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Neoadjuvant chemotherapy of BC raises the rate of breast conserving surgery but the influence upon survival remains to be shown. Reaching a pCR with preoperative treatment might influence survival positively. To date, no predictive factor associated with the chance to reach a pCR with neoadjuvant chemotherapy is established. Thus, we studied prospectively the potential value of the radiological tumor stage (T),pretreatment clinical nodal stage (N),menopausal status (MS),hormone-receptor status (HR),number of treatment cycles (Tx),p53-status (p53),and HER2-status (HER) in 65 consecutive patients with BC treated neoadjuvantely with epirubicin and docetaxel+G-CSF (ED+G). The results of the univariate and the multivariate analysis of all investigated parameters are shown in the table.

	n =	stratum	n (%)	pCR-rate: n(%)	p univariate	p multivariate
T-status	65	1+2 vs. 3+4	27(42) vs. 38(58)	5(19) vs. 5(13)	ns	ns
N-status	65	pos. vs. neg.	34(52) vs. 31(48)	3(9) vs. 7(16)	ns	ns
MS	65	prae vs. post	31(48) vs. 34 52)	6(19) vs. 4(12)	ns	ns
HR	65	pos. vs. neg.	38(58) vs. 27(42)	7(18) vs. 3(11)	ns	ns
Tx	65	2-4 vs. 5-8	21(32) vs. 44(68)	4(19) vs. 6(14)	ns	ns
p53	44	pos. vs. neg.	19(43) vs. 25(57)	5(26) vs. 4(16)	ns	not done (low n)
HER	57	pos. vs. neg.	13(23) vs. 44(77)	8(62) vs. 2(5)	< 0.0001	< 0.0001

As shown, neither the clinical, nor the biological factors investigated were correlated with pCR. Not even the number of treatment cycles showed any correlation with the chance to experience a pCR. Only the HER2-status which was positive in 23% of the patients demonstrated to be an independent predictive parameter strongly associated with the chance to reach a pCR after treatment with ED+G. These data supports the hypothesis that HER2-positivity might be correlated with the sensitivity or resistance to certain cytostatics and hormonal compounds. Our results clearly show that future randomized trials in the neoadjuvant setting of BC must include the HER2-status as a stratum to avoid major flaws in the interpretation of the study outcome. Furthermore, since a pCR-rate of 62% of HER2-positive patients is amongst the highest ever reported, our results may serve for the design for planned clinical studies.

24 Phase II Trial of Herceptin Induction Followed by Combination Therapy with Paclitaxel and Carboplatin: A Minnie Pearl Research Network Trial.

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A survival benefit has been noted with Herceptin in combination with chemotherapy in patients (pts) with metastatic breast cancer. A phase II trial was initiated to evaluate the activity of Herceptin as a single agent and the benefits of adding paclitaxel and carboplatin. Eligibility criteria included 2 or 3+ Her-2-neu overexpression, first-line therapy for metastatic disease, and normal organ function. Herceptin 8mg/kg was given as a loading dose, followed by 4mg/kg weekly. After 8 wks, pts were evaluated and those with a minor response or better continued an additional 8 wks of Herceptin. Pts with stable disease began chemotherapy with paclitaxel (70mg/m2/week) and carboplatin (AUC 2.0/week) in combination with Herceptin (2mg/kg) weekly for 6 wks with cycles repeated q8wks. Pts with disease progression at evaluation dc'd Herceptin and began paclitaxel and carboplatin on the weekly dosing schedule. To date, 37 pts are evaluable [median age 52 (range 39-77), PS 0 = 13, PS 1 = 22, PS 2 = 2]. 15 pts were ER+ and 19 had prior adjuvant therapy (13 prior doxorubicin). Her-2 overexpression (DAKO kit) was 3+ in 16 and 2+ in 21 pts. 29 pts (78%) completed 8 wks of induction Herceptin without evidence of progression. At the 8-wk evaluation, 1 CR and 5 PRs were noted (21% response rate), with 3 minor responses. These pts received an additional 8 wks of Herceptin prior to starting combination therapy. Currently, 12 pts have proceeded to paclitaxel, carboplatin, & Herceptin therapy and are evaluable for response. Two CRs and 5 PRs were noted (58% RR). Of the 8 pts progressing on induction Herceptin, 4 PRs were confirmed (50% RR) with subsequent paclitaxel/carboplatin. Brief and reversible myelosuppression was the primary toxicity encountered. No significant non-hematologic toxicities were observed. Significant LVEF declines were noted in two pts, 25% (63 to 38%) and 17% (46 to 29%). No symptomatic cardiotoxicity was observed. In summary, Herceptin has activity as a single agent in first-line therapy of metastatic breast cancer. Additional responses are seen in pts with stable disease with the addition of paclitaxel and carboplatin. The combination of paclitaxel and carboplatin also has activity in pts progressing on Herceptin.

25 Treatment Beyond Progression in the Herceptin Pivotal Combination Chemotherapy Trial.

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Study H0659g was designed to provide, especially for those patients randomized to the chemotherapy alone treatment group, an extended period of access to Herceptin after participation in the pivotal combination chemotherapy trial, H0648g. Patients entered after documented progression in the pivotal trial and then received Herceptin alone or in combination with chemotherapy at the discretion of the treating physician. Of 235 patients in the Herceptin plus chemotherapy (H→H) group, 93 (40%) entered study H0659g, while 154 of 234 (66%) patients from the chemotherapy alone group (C→H) entered the trial. Approximately ¾ of all patients expressed HER2 at the 3+ level by IHC. Most patients had visceral involvement. 76% of H→H patients and 68% of C→H patients received chemotherapy. 55% of H→H patients and 76% of C→H patients experienced an adverse event. Most events were mild to moderate in severity. Infusion reactions were seen more frequently in the C→H group. 16 patients (14 from C→H) developed cardiac dysfunction. All had prior anthracycline exposure. Responses were seen in 11% of patients in the H→H group and 14% of patients in the C→H group. Clinical benefit rates (CR+PR+SD>6 mos.) were 22% and 32%, respectively. Responses to single agent Herceptin were seen in both groups. Other than single agent use, the most frequent treatment at the time of response was paclitaxel and Herceptin. Other combinations also produced responses. 80% of responders in the H→H group and 100% of responders in the C→H group overexpressed HER2 at the 3+ level by IHC. Response duration was 6.7 months in the H→H group, and 7.4 months in the C→H group. These results suggest that patients with progressive metastatic breast cancer following one course of treatment, including Herceptin plus chemotherapy, may respond to a subsequent course of Herceptin containing-therapy. Herceptin use beyond disease progression should be examined in well controlled trials.

26 Effects of ZD 1839 (Iressa™), a Novel EGF Receptor Tyrosine Kinase Inhibitor, on Breast Cancer Cell Proliferation and Invasiveness.

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Activation of the epidermal growth factor receptor (EGFR) and related receptors such as erbB2 promotes growth and invasiveness via multiple signalling pathways. Overexpression of these receptors is associated with poor prognosis in breast and ovarian cancer. Novel drugs that target these receptors therefore have great potential therapeutic benefit. ZD1839 (Iressa™) is an orally active, selective EGFR-TKI (EGFR tyrosine kinase inhibitor) that blocks signal transduction pathways implicated in proliferation and survival of cancer cells. Here, we have tested the effects of ZD1839 on a range of cancer cell lines that exhibit various degrees of EGFR and erbB2 overexpression. ZD1839 inhibited proliferation (in 10% FCS) in all cell lines tested in vitro, as follows:

cell line	EGFR	erbB2	Inhibition (%) at 1 μM	IC_{50} (nM)
MDA-MB231	+++	+	50 ± 2 (3)	7
SKBR3	++	+++	53 ± 2 (3)	40
SKOV3	+	+++	51 ± 5 (3)	19
BT474	+	+++	27 ± 20 (3)	28
T47D	+	+	16 ± 4 (3)	90

Growth in nude mice of MDA-MB231 and SKOV3 cells was significantly ($p<0.05$) inhibited by ZD1839 (75mg/kg). ZD1839 also reduced EGF-induced activation of phospholipase Cγ1, protein kinase B and ERK MAP kinase, and significantly ($p<0.05$) inhibited EGF-stimulated uPA production by 52 ± 5% (n=3) in MDA-MB-231 cells in vitro.

Thus ZD1839 shows anti-proliferative activity in cancer cells that overexpress the EGFR, and also in cancer cells overexpressing erbB2. ZD1839 therefore shows promise as a novel form of therapy for cancers overexpressing these receptors.

27 Zoledronic Acid Reduces Skeletal Related Events in Patients with Osteolytic Metastases: A Double-Blind, Randomized Dose Response Study.

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This study evaluated zoledronic acid (ZOL), a new high potency bisphosphonate, given as a 5 min infusion in patients with malignant osteolytic disease. 280 breast cancer or multiple myeloma patients were randomized to blinded treatment with either 0.4 mg (68 pts), 2.0 mg (73 pts) or 4.0 mg (66 pts) ZOL administered as a 5 minute infusion or 90 mg pamidronate (PAM) (73 pts) administered as a 2 hr infusion, every 4 wks for 10 months. The primary endpoint was the proportion of patients receiving radiation to bone. Also measured were skeletal related events (SREs) (pathological fractures, spinal cord compression, surgery/ radiation to bone, hypercalcemia), bone mineral density, bone markers, ECOG, pain/analgesic scores, and safety. The proportions of pts receiving radiation to bone were 24%, 19% and 21% in the ZOL 0.4, 2.0, and 4.0 groups, respectively, and 18% in the PAM 90 mg group. The 2.0 and 4.0 ZOL and the PAM 90 group met the primary efficacy criterion of significantly < 30% of pts having radiation to bone ($p<0.05$), but Zol 0.4 mg did not ($p=0.104$). 35, 33 and 30% of Pts in the ZOL 2.0, ZOL 4.0 and PAM 90 groups, respectively, had a least one SRE, vs 46% for ZOL 0.4 ($p<0.05$ for PAM/ZOL 0.4). Median times to first event were 167, 175, 231 and 254 days for Zol 0.4, 2.0, 4.0 and PAM 90, respectively ($p<0.05$ for PAM/ZOL 0.4). Zol 0.4 was also significantly inferior to ZOL 4.0 in increase in bone mineral density and deceases in bone markers. Adverse events (AEs) were similar between the ZOL and PAM groups, with skeletal pain, fatigue, nausea, vomiting and headache the most common AEs reported.

Summary: A 5 minute infusion of 2.0 to 4.0 mg zoledronic acid was at least as effective as a 2 hr 90 mg pamidronate infusion in the treatment of osteolytic metastases. ZOL 0.4 mg did not meet the primary criteria for efficacy, and was inferior to ZOL 4.0 mg on a number of secondary efficacy parameters. Zoledronic acid 4.0 mg should be the dose evaluated further in the treatment of bone metastases.

28 Clinical Activity with the Farnesyl Transferase Inhibitor R115777 in Patients with Advanced Breast Cancer – Relationship with Tumour Phenotype.

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Signal transduction inhibition represents a novel therapeutic strategy for the treatment of breast cancer. The ras family of proteins are essential components of the mitogenic signalling pathway, and although oncogenic mutations are rare in breast cancer abnormal growth factor activation (HER-2 amplification or EGFr overexpression) may still operate through ras dependent pathways. Ras is synthesised as a cytosolic precursor that localises to the inner plasma membrane following post-translational addition of a 15-carbon farnesyl isoprenoid moeity. R115777 is an orally active non-peptidomimetic farnesyl transferase inhibitor (FTI) which specifically blocks isoprenylation and activation of proteins, including p21 ras. In pre-clinical studies R115777 inhibited the growth of MCF-7 breast cancer cells and xenografts in a dose dependent manner. From phase I studies, a continuous oral dosing schedule was developed with trough plasma concentrations consistent with the IC50. We have treated 39 patients with advanced breast cancer in the first phase II study of R115777. Median age was 58 years (range 32-79); Prior therapy for advanced disease included second-line hormonal therapy in 25 (64%) patients and/or one chemotherapy regimen in 18 (46%) patients. Tumour phenotype was; 17 ER+ve, 12 ER-ve, 10 ER unknown, and 14 HER-2+ve, 10 HER2-ve, 15 unknown. Confirmed partial responses were seen in 4 (10%) patients, both at visceral and soft tissue sites. Median duration of response was 7 months (range 5-9+). A further 10 (26%) patients had stable disease at the 3 month evaluation, 3 of whom have been stable for > 6 months. Preliminary analysis suggests that response occurred independent of HER-2 or ER status. Treatment was well tolerated, with myelosuppression the most frequent toxicity; at 300 mg b.i.d. 42% developed grade 3/4 neutropenia after a median of 32 days with recovery occurring over 1-2 weeks. Grade 2/3 parasthesia/numbness occurred in 12 (31%) patients after an average of 8 weeks therapy. These initial results suggest that R115777 has clinical activity in advanced breast cancer, and further updated data on the relationship of response to tumour phenotype (including EGFRr expression and ras mutation status) will be presented.

29 Morphometric and Cytogenetic Investigations in In Situ Carcinomas of the Breast.

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The reproducibility of the established protocols for the classification of pre-invasive breast disease is poor. Classification could be better founded using biological markers which could increase reproducibility. Especially the classification of ductal carcinoma in situ (DCIS) and to a lesser extent lobular carcinoma in situ (LCIS) still is a matter of debate.

To gain insight in the relationships between distinct cytogenetic alterations in DCIS/LCIS and reproducible quantitative features as measures of differentiation, proliferation and apoptosis we investigated 57 breast carcinomas in situ by means of Comparative Genomic Hybridization (CGH) and correlated the cytogenetic findings with the mean nuclear area (MNA), the presence or absence of necrosis, and the mitotic (MI) and apoptotic index (AI).

Loss of 8p and gains of 8q and 6q were respectively associated with a significantly higher MI and AI in contrast to tumours with a loss of 16q which was associated with a lower MI and AI. A significantly higher number of alterations per case and an increased rate of tumours with amplifications were seen in tumours with gains of 6q, 8q, 17q and tumours with loss of 13q. The loss of 16q and the gain of 17q, correlated with the absence or presence of necrosis, respectively.

Our data clearly demonstrate that distinct cytogenetic changes correlate with phenotypic changes (necrosis, nuclear area), proliferation (mitotic index) and apoptosis (apoptotic index). This further substantiates recently proposed pathways in the pathogenesis of breast cancer. These data may be used to refine existing classification schemes and contributes to the understanding of breast pathogenesis.

30 Ductal Invasive G2 and G3 Carcinomas of the Breast Are the End Stage of at Least Two Different Lines of Genetic Evolution.

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Ductal invasive G2 and G3 carcinoma represent the majority of invasive breast cancer. Previous morphological and cytogenetic studies have provided evidence that ductal invasive G2 carcinoma may originate from at least two different genetic pathways. The aim of this study was to further evaluate the heterogeneity of G2 breast cancer in comparison with G3 cancers by cytogenetic and quantitative analysis.

To this end, we investigated 35 cases of ductal invasive G2 and 42 cases of ductal invasive G3 carcinomas by means of Comparative Genomic Hybridization (CGH) and correlated these findings with the DNA ploidy status, the mitotic activity index (MAI), the mean nuclear area (MNA), the volume per lumen (VPL) and clinico-pathological parameters.

Our findings demonstrate that ductal invasive G3 carcinomas represent a rather homogeneous genetic tumour group. In contrast, ductal invasive G2 carcinoma have to be interpreted as the morphological end stage resulting from two different cytogenetic pathways with the loss of 16q-material as the cytogenetic key event in the evolution of a subgroup of this entity. By correlating the genetic alterations with the DNA ploidy status, the proliferative activity and parametrs of nuclear pleomorphism, an extended morphology based cytogenetic progression model with early and late genetic alterations in the pathogenesis of breast cancer is presented. The correlation with the MAI gives rise to the hypothesis that these different genetic pathways also differ in the proliferation rate which is determined early in breast pathogenesis. Further studies have to elucidate which genes contribute to an altered proliferation rate in these subgroups and the associated prognosis.

31 A Hypersensitive Estrogen Receptor α Protein in Premalignant Breast Lesions.

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The best current model of breast cancer evolution suggests that most cancers arise from certain premalignant lesions. We have identified a common (34%) somatic mutation in the estrogen receptor (ER) α gene in a series of 59 typical hyperplasias, a type of early premalignant breast lesion. The mutation, which affects the border of the hinge and hormone binding domains of ERα, showed increased sensitivity to estrogen as compared to wild-type ERα in stably transfected breast cancer cells, including markedly increased proliferation at subphysiologic levels of estrogen. The mutated ERα exhibits enhanced binding to the SRC-1 family of co-activators at low levels of hormone, which may partially explain its increased estrogen responsiveness. This data suggests that this mutation may promote or accelerate the development of cancer from premalignant breast lesions. To examine this hypothesis we have generated transgenic mice expressing of the mutated ER under the control of the MMTV promoter which directs expression to the breast. Preliminary results in 2 and 4-month old virgin females suggest that expression of this mutant ER results in a unusual phenotype with the appearance of distended ducts, and a "tangled" growth pattern. There appears to be reduced side budding in the transgenic animals, suggestive that differentiation could be possibly delayed or inhibited in these animals. Of course, longer studies will be necessary to follow this unusual phenotype. Since premalignant breast lesions are microscopic masses with a positive growth imbalance, the hypersensitive ERα mutation could be an important factor contributing to this imbalance. If there is indeed a correlation between risk and expression of this ERα mutation, genetic analysis for the mutation in premalignant lesions might help to identify patients who would benefit from preventive measures.

32 Inhibition of Breast Cancer Cell Migration by Insulin-Like Growth Factor Binding Protein-1 (IGFBP-1).

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Insulin-like growth factor-I (IGF-I) stimulates proliferation in some breast cancer cell lines. We have shown that recombinant human IGF binding protein-1 (rhBP-1) can neutralize IGF action and inhibit these proliferative effects. In addition to stimulating cell growth, IGF-I induces cell migration in some breast cancer cells. In this study we tested the ability of rhBP-1 to inhibit IGF-mediated cell migration. Treatment of F-11 cells, a metastatic variant of MDA-MB-231 cells, with 5nM IGF-I results in cell migration as assayed in a modified Boyden chamber assay in serum-free media (SFM). IGF-I treatment results in phosphorylation of insulin-receptor substrate-2 (IRS-2) which subsequently activates multiple downstream signaling pathways. To test the contribution of type I IGF receptor (IGF1R) activation in cell migration, we found that αIR-3, a monoclonal antibody that blocks IGF1R activation, inhibited IGF-mediated cell migration. When cells are incubated in 40nM rhBP-1, the stimulatory effect of IGF-I on cell migration is also eliminated. Like αIR-3, rhBP-1 also decreased IGF-mediated phosphorylation of IRS-2. Unexpectedly, in the absence of IGF-I, rhBP-1 inhibited F-11 cell migration to levels below untreated cells. Since SFM contains fibronectin and rhBP-1 contains an Arg-Gly-Asp (RGD) motif involved in binding fibronectin receptors such as α5β1 integrin, it was possible that rhBP-1 influenced integrin function. A synthetic peptide that blocks fibronectin binding to its receptor (GRGDTP) also inhibited the IGF migration effects, while control RGE peptides did not. RGD peptide also prevented activation of IGF1R by IGF-I in F-11 cells. In contrast, activation of IGF1R in MCF-7 cells, which are not stimulated to migrate by IGF-I, were not affected by RGD peptide. We conclude that integrin occupancy may be required for cell migration mediated by IGF-I. In the absence of integrin occupancy, IGF1R activation did not occur in these migrating cells. In addition, inhibition of cell migration by rhBP-1 may be due to dual effects; neutralization of IGF action and interruption of integrin function.

33 TGF-β Stimulates Tumor Production of PTHrP Via Smad and MAP Kinase Signaling Pathways.

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Substantial data support central roles for bone-derived TGF-β and tumor-derived PTHrP in a vicious cycle of local bone destruction in osteolytic metastases. TGF-β, released from bone during osteoclastic resorption, stimulates PTHrP production by tumor cells. PTHrP in turn mediates bone destruction by stimulating osteoclasts. However, the signaling pathways by which TGF-β increases PTHrP secretion by tumor cells are unknown. TGF-β mediates its effects via cell-surface receptors to the intracellular Smad proteins, but there is also evidence for other signaling pathways. To determine the role of the Smad proteins, we stably expressed wild type and dominant-negative mutants of Smads 2, 3, and 4 into MDA-MB-231 cells. The lines were characterized for PTHrP production in response to TGF-β and for signaling by transient transfection with the TGF-β-responsive 3TP-Lux reporter and luciferase assays. Compared to empty vector controls, overexpression of wt Smads 2, 3 and 4 enhanced both PTHrP production and 3TP-Lux luciferase activity in response to TGF-β while the dominant negative TGF-β receptor-expressing cells were unresponsive to TGF-β in both assays. In contrast, dominant-negative Smads [Smad2(3S-A), Smad3(3S-A), Smad3(D407E), Smad4(1-514)] reduced, did not totally suppress, TGF-β-stimulated PTHrP secretion. The results suggested both Smad-dependent and -independent TGF-β signaling pathways. To examine Smad-independent TGF-β signaling, MDA-MB-231 cells were treated with inhibitors of MAP kinase pathways as well as protein kinases C, A and G, and PI3 kinase. Two specific inhibitors of the p38 MAP kinase pathway significantly reduced both basal and TGF-β-stimulated PTHrP production in parental cells. The combination of Smad2 or Smad3 dominant-negative blockade and p38 MAP kinase inhibition resulted in greater inhibition of TGF-β-stimulated PTHrP production than either modality alone. The MEK inhibitor reduced basal PTHrP production by parental MDA-MB-231 cells as well as basal and TGF-β-stimulated PTHrP production in those that expressed dominant-negative Smads. Other used inhibitors had no effect on PTHrP production. In sum these data support both Smad-dependent and -independent pathways for the TGF-β stimulation of PTHrP production by breast cancer cells. The MAP kinase pathways appear to be a major component of this Smad-independent signaling by TGF-β and represent new molecular targets for anti-osteolytic therapy.

34 Antigen Presentation Prevents Tumor Development in Tumor Challenged Her-2 Transgenic Mice.

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Introduction: Antigen presenting cells (APC) play a crucial role in the generation of T cell-mediated immune responses. Effective antitumor immunity will require both antigen presentation by APC and T cell effector activation. Tumor cells are poor APCs for two major reasons; they barely express Major Histocompatibility Complex Antigens and they lack the expression of costimulatory molecules. We asked whether ex-vivo modified tumor cells that express MHC class II determinants and B7-1 costimulatory molecule can be effective APCs. Methods: Breast cancer tumor cell lines generated from the mouse Her2/neu+ transgenic tumor were transduced ex-vivo to express IFN-γ, CIITA or B7-1 costimulatory molecules using a retroviral delivery system. Syngeneic Her2 transgenic mice were injected with unmodified tumor cells or modified cells that expressed either CIITA, IFN-γ, or B7-1 at 8-10 weeks of age, 6animals per group. Results: Tumor grew in 6/6 mice in the control and IFN-γ group. Although the time lapse necessary to develop palpable tumor growth was comparable in both groups, mice injected with IFN-γ modified cells exhibited extensive inflammation at the site of injection with swelling and redness around the growing tumor and two animals presented lung metastases and died before the end of the study (as did one in the control group). In sharp contrast, smaller tumors grew in 5/6 mice vaccinated with CIITA modified cells, and only one out of 6 mice vaccinated with B7-1 expressing cells developed tumor at injection site after five months of observation. Three out of six B7-1 injected mice developed spontaneous mammary tumors at 10 months of age and 3/6 stayed tumor free (90% of animals in the Tg colony, develop tumors by 10 months of age). Conclusions: Immunization with B7-1 and possibly CIITA expressing cells my be a potent immunotherapy for breast cancer and deserves further investigations. The differential response between the two groups (IFN-γ and B7-1) might represent different T helper type responses, thus modulating pathogenic humoral immune response. Modified tumor cells expresing both CIITA and B7-1 are under study.

35 Risk of Ipsilateral Recurrence (IBTR) and Metachronous Contralateral Breast Cancer (CBC) after Breast Conservation Therapy (BCT) in Women with Germline BRCA1 or BRCA2 Mutations.

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An anonymized retrospective design was employed to study Ashkenazi Jewish women undergoing BCT at 2 institutions to determine clinical outcomes in women with BRCA mutations. Clinical data and follow-up were collected and DNA samples obtained from archived materials. After irretrievable anonymization, DNA was tested for the Ashkenazi founder mutations BRCA1 185delAG, BRCA1 5382insC, and BRCA2 6174delT. There were 466 women undergoing BCT for 502 cancers. 53 women (11.4%) were found to have a BRCA mutation (41 BRCA1, 11 BRCA2, 1 BRCA1 and BRCA2). 65/502 (12.9%) cancers were associated with mutations. BRCA-associated cancers (BAC) were more likely to be diagnosed before the age of 50 (P<0.001), but tumor stage was not different from non-BAC. With median follow-up of 103.4 months, IBTR occurred in 8/65 BAC and 34/437 non-BAC. The hazard ratio (HR) for IBTR in BAC was 2.2 (95% CI: 1.0-4.8). The risk of IBTR at 60 and 103.4 months was 13% and 13%, respectively, vs. 5% and 7% in non-BAC (P=0.07). A total of 459 women were at risk for contralateral cancer (7 had previously undergone contralateral mastectomy). With a median follow-up of 105.8 months, CBC occurred in 13/52 women at risk with a BRCA mutation and 30/413 women at risk who did not have a mutation. The HR for CBC in women with mutations was 4.7 (95% CI: 2.4-9.1). CBC risk at 60 and 105.8 months was 14% and 31%, respectively, v. 3% and 7% in women without mutations (P<0.0003). Based on these results, BCT does not appear to be contraindicated in BAC, despite a modestly increased risk of IBTR. Contralateral risk, however, is significantly greater in women with mutations.

36 A Randomised Study of Sector Resection with and without Radiotherapy for Women with Node-Negative Stage I and II Breast Cancer.

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Breast conserving surgery in Sweden is performed with sector resection according to common Swedish guidelines. A previous Swedish trial, the Uppsala-Örebro trial, suggested that subgroups of patients could be spared postoperative radiation therapy. Since the previous study public mammography screening has been implemented in all Sweden. The purpose of the present trial was to study sector resection with and without radiotherapy in an extended trial with patients recruited form three health care regions.

From January 1991 to October 1997, 1187 women with radically resected stage I and II node-negative breast cancer were randomised to: postoperative radiotherapy, 50 Gy in 25 fractions during five weeks (arm A, 593 patients), or no postoperative radiation (arm B, 594 patients). Median age was 60 years, and median tumour size was 12 mm for all randomised patients. 65% of all cases were detected by mammography screening.

Numbers of first events in the two arms, A vs. B, were as follows: local recurrence in the breast 26 vs. 78, axillary recurrence 3 vs. 7, distant metastases 24 vs. 24, and contra-lateral breast cancer 17 vs. 17. Median follow up was 60 months among disease-free patients. With a follow-up for survival up to December 31,1999, forty-eight patients in arm A and 57 in arm B died. An extended analysis will be presented.

37 Regional Nodal Failure Patterns Following Mastectomy without Radiation.

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Indications for postoperative regional nodal irradiation following definitive surgery for breast cancer remain controversial. The purpose of this study was to determine regional nodal failure patterns in order to define subgroups of patients who might benefit from adjuvant regional nodal irradiation. The cohort consisted of 1,031 patients treated with mastectomy (including a level I-II axillary dissection) and doxorubicin-based systemic therapy without radiation on five clinical trials at M.D. Anderson Cancer Center for Stage II-III breast cancer. Patient records, including pathology reports were retrospectively reviewed. All regional recurrences, including isolated failures as well as failures with or without distant metastasis were recorded. Median follow-up was 116 months (range 6-262 months).

Twenty-five patients recurred within the dissected axilla (10-year actuarial rate 3%). Of these, 17 were isolated regional failures. The risk of failure in the dissected axilla was not significantly higher for patients with increasing numbers of involved nodes, increasing percentage of involved nodes, larger nodal size or gross extranodal extension (≥2mm). Only 3/100 patients with <10 nodes examined recurred in the dissected axilla.

Seventy-five patients experienced a recurrence in the axillary apex(Level III)/ supraclavicular fossa (10-year actuarial rate 8%). Forty-seven were isolated recurrences. Significant predictors of failures in this region included ≥4 involved axillary lymph nodes, ≥20% involved axillary nodes, and the presence of gross extranodal extension (10-year actuarial rates 15%, 14%, and 20% respectively, $p<0.01$). The extent of axillary dissection or the size of the largest involved node were not predictive of failure within this region.

These results, in contrast to those of the Danish 82b trial, suggest that failure in the dissected axilla is an uncommon occurrence and that supplemental radiotherapy to the axilla is rarely warranted. However, patients with ≥4 involved axillary lymph nodes, ≥20% involved axillary nodes, or gross extranodal extension are at increased risk of failure in the axillary apex/ supraclavicular fossa and should receive radiation to these regions as well as to the chest wall.

38 Loco-Regional Radiation for High Risk Breast Cancer - Results of Short Fractionation.

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A recent randomized trial (Whelan et al, ASCO 2000) demonstrated that radiation therapy (RT) with 42.5 Gy in 16 fractions is equivalent to 50 Gy in 25 fractions after lumpectomy. The current study evaluated rates of local and regional recurrence in high risk breast cancer patients after combined local and regional RT with short fractionation.

Subjects were 1,142 patients who were under 90 years of age, did not have stage N2, N3 or M1 disease at diagnosis who were treated at the British Columbia Cancer Agency between 1989 and 1995 with loco-regional RT following lumpectomy or mastectomy. All patients had an axillary node dissection and 95.2% received systemic therapy.

Stages at presentation (AJC/UICC 1987) were 25.8% T1, 40.5% T2, 11.2% T3 and 5.6% T4 (16.6% were unknown). Axillary nodes were positive in 90.4% of cases. RT doses to the breast or chest wall were either 44 Gy or 40 Gy in 16 fractions for 91.6% of patients. RT doses to the regional nodes were 35-44 Gy in 16 fractions for 95.4% of patients.

The actuarial local recurrence rate (ipsilateral breast or chest wall) was 7.5% at 5 years and 9.2% at 8 years. The actuarial regional recurrence rate (ipsilateral axillary, supraclavicular, infraclavicular or internal mammary areas) was 6.9% at 5 years and 7.6% at 8 years. The actuarial rate of distant failure was 30.5% at 5 years and 40.3% at 8 years. Event-free survival at 5 years was 58.7% and overall 5 year survival was 68.9%. Rates of significant arm lymphedema were low (<5%) and there were no reported cases of radiation brachial plexopathy.

Locoregional radiation using short fractionation with just 16 daily fractions in women with positive nodes and/or high T stage was well tolerated and effective in achieving loco-regional control rates comparable to rates reported in series using longer fractionation.

101 The Almanac Trial: Initial Experience.

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Introduction: In recent years sentinel node technique has been used increasingly in early breast cancer. However, there are no published randomised controlled trials to validate this technique in breast cancer. The ALMANAC (Axillary Lymphatic Mapping Against Nodal Axillary Clearance) trial is a multi-centre randomised controlled trial comparing sentinel node biopsy with standard axillary therapy in early breast cancer.

Methods: The trial, comprises an audit phase to assess the learning curve and a randomised phase. In the audit phase all patients had a sentinel node biopsy performed prior to a definitive axillary procedure. The primary objective of the audit phase is to measure the learning curve and standardise this new surgical technique. In order to proceed to the randomised phase, a localisation rate of >90% and a false negative rate of 5% or less are required in 40 consecutive patients per surgeon.

The sentinel node was localised using a combination of blue dye and radioactive isotope with a mandatory pre operative scintiscan.

Results: There are currently 14 centres across the UK with 24 surgeons participating in the audit phase of the trial. The early results in 292 patients show that 30% (89/292) of cancers were screen detected and the tumour size ranged from 2 to 100 mm (mean 21.2 mm, sd 11.8).

Axillary drainage was seen in 75% of cases and internal mammary drainage in 10%. A total of 631 sentinel nodes were removed, an average of 2.2 per patient (range 1-8). Of these nodes there were 483 (78%) blue nodes and 516 (86%) hot nodes. A sentinel node was identified in 277 (95.5%) patients. The mean time for performing a sentinel node biopsy was 17.5 minutes (range 2-75 minutes).

37.3% of patients were node positive. The false negative rate was 7%.

Conclusions: The initial results of the ALMANAC trial confirm the feasibility of sentinel node biopsy as an axillary staging procedure in the management of breast cancer. The randomised phase is underway and will demonstrate if sentinel node biopsy has any advantage over conventional axillary treatment in terms of local control, morbidity, quality of life and health economics.

102 Physiologic and Socioeconomic Consequences of Breast Cancer Surgery: A Comparison between Sentinel Lymph Node Biopsy and Axillary Lymph Node Dissection.

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Background: Sentinel lymph node biopsy (SLNB) is an emerging technology in the treatment of breast cancer. This study was designed to compare the postoperative morbidity and socioeconomic impact of SLNB versus routine axillary lymph node dissection (ALND) in patients with early stage breast cancer.

Methods: A prospective, non-randomized, controlled study was designed to include all patients who underwent breast conservation surgery and SLNB±ALND. Group A consisted of patients who had a negative SLNB and did not go on to completion ALND. Group B (control group) consisted of patients who underwent a SLNB followed by a completion ALND (level I and II) because either, 1) their sentinel node contained cancer, or 2) they were within the validation phase of our sentinel lymph node protocol. All patients were assessed at a minimum of 6 months from the completion of breast irradiation with a questionnaire addressing subjective complaints of arm symptoms and recovery time. Patients then underwent a standardized physical examination to determine arm circumference.

Results: Data was obtained from 96 patients with a mean follow-up of 15.3 months (range 8-29 months). Groups A (n=48) and B (n=48) were comparable with respect to age and comorbidity. The difference in mid-bicep circumference for Group A and Group B was significant when comparing the ratio of procedure arm to the non-procedure arm and when subtracting the non-procedure arm from the procedure arm ($p<0.003$ and $p<0.016$, respectively). Return to normal activity in less than 4 days was reported in 70.7% of Group A, compared with 7.1% of Group B ($p<0.001$). Other objective comparisons are listed in the table below.

Conclusion: SLNB results in less postoperative morbidity in terms of subjective arm numbness, objective mid-arm swelling and the requirements for a wound drain. Expeditious return to work or normal activity following SLNB has potentially significant socioeconomic consequences.

	Arm Complaints	Arm Numbness	Drain Days/pt	Outpatient Procedure
Group A	41.7%	16.7%	0.5	87.5%
Group B	87.5%	81.2%	13.2	14.6%
p value	<0.001	<0.001	<0.001	<0.001

103 Quantitative Real-Time RT-PCR Detection of Breast Cancer Micrometastasis Using a Multi-Gene Marker Panel.

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The presence of metastatic disease in the ipsilateral axillary lymph nodes (ALN) remains the most valuable predictor for breast cancer recurrence. However, staging by ALN dissection followed by H&E staining apparently lacks sensitivity since up to 30% of patients with pathologically negative nodes develop recurrent disease. Real-time RT-PCR is a relatively new technology that uses an on-line fluorescence detection system to determine gene expression levels. It has the potential to significantly impact detection of breast cancer metastasis by virtue of its exquisite sensitivity, high-throughput capacity and quantitative readout system. To assess the utility of this technology in breast cancer staging, we determined the relative levels of expression of 11 cancer-associated genes (*MAM*, *PIP*, *CEA*, *CK19*, *VEGF*, *Erb2B*, *MUC1*, *c-myc*, *p97*, *VIM*, and *Ki67*) in 42 negative control normal lymph nodes and in 16 histopathology-positive ALNs. We then performed a receiver operating characteristic (ROC) curve analysis to determine the sensitivity and specificity levels of each gene. At a 98% level of specificity, the following levels of sensitivity were observed: *MAM*, 100%; *PIP*, 68%; *CK19*, 67%; *MUC1*, 51%; *CEA*, 41%; and *Erb2B*, 38%. Areas under the ROC curves indicated that the most accurate diagnostic markers were *MAM* (100%), *CK19* (91%), *PIP* (87%), and *CEA* (87%). *MAM* was overexpressed in the pathology-positive ALNs at levels ranging from 14- to 1.5 x 10^5-fold above normal mean expression, whereas *PIP* was overexpressed from 52- to 1.3 x 10^6-fold above normal. Analysis of 21 pathology-negative lymph nodes revealed overexpression of at least one of three genes (*MAM* (3 nodes), *PIP* (4 nodes), and *CEA* (1 node); overexpression range = 25- to 5.5 x 10^4-fold above normal) in 6 of 21 nodes (29%). These results provide evidence that the most accurate diagnostic markers for detection of breast cancer metastatic disease in ALNs are *MAM*, *PIP*, *CK19*, and *CEA*, and that these markers should be applied as a panel for detection of micrometastatic disease. A multi-institutional trial is currently ongoing to correlate gene overexpression with clinical outcomes.

104 Detection of Axillary Node Positivity Is Increased with Sentinel Lymph Node Biopsy in Early Stage Breast Carcinoma.

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Axillary lymph node status is the single most important prognostic factor for patients with breast carcinoma. The detection of positive nodes significantly impacts patient treatment, particularly for patients with tumors ≤ 1 cm who otherwise would not receive systemic therapy. Sentinel node biopsy (SNB) with its enhanced pathology has been reported to increase detection of node positivity over axillary dissection (AD). We compared the incidence of axillary node positivity found with AD to that of SNB for patients with microinvasive, T1a, and T1b breast carcinoma. Between January 1, 1989 and December 31, 1991 258 patients were identified with tumors≤1 cm who underwent standard AD obtaining a minimum of 10 nodes. Between June 30, 1997 and November 19, 1999 596 patients were identified with 603 tumors ≤1 cm who underwent SNB. For each tumor category, there was no statistically significant difference between the SNB group and the AD group with respect to patient age, tumor location, laterality, type, histologic grade, or the presence of lymphovascular invasion. However, there was a statistically significant difference between the two groups with respect to multifocality, palpability, estrogen receptor (ER) status and frequency of breast conservation surgery. The table below lists the incidence of node positivity for each tumor category comparing the two different techniques for axillary evaluation.

	Microinvasive	**T1a**	**T1b**	**All**
AD	0/13 (0.0%)	8/68 (11.8%)	26/177 (14.7%)	34/258 (13.2%)
SNB	2/28 (7.1%)	30/180 (16.7%)	91/395 (23.0%)	123/603(20.4%)
p value	p= 1.0	p= 0.43	**p=0.025**	**p=0.012**

The detection of node positivity was increased with SNB for all categories, and was statistically significantly increased overall and specifically for those with T1b tumors. Among patients with positive nodes who underwent SNB, nodal positivity was detected by immunohistochemistry alone in both patients with microinvasive tumors (100%), 13/30 with T1a tumors (43.3%), and 26/91 with T1b tumors (28.6%). SNB significantly increases the detection of nodal positivity in patients with the earliest breast cancers, and should be offered to these patients by surgeons experienced with the technique.

105 The First 2,000 Cases - Axillary Node Metastases in the Sentinel Lymph Node Era.

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The presence of axillary lymph node metastases (ALNM) remains the most important prognostic indicator for a woman with breast carcinoma (ca). The adoption of sentinel lymph node (SLN) biopsy and enhanced pathologic analysis of the SLN marks a new era in the treatment of breast cancer. We undertook this analysis to examine the clinical and pathological predictive factors of ALNM in this new era.

METHODS: From 9/1996 to 3/2000, we performed 2,000 consecutive SLN procedures (cases) in 1,819 patients. 1,849 cases had measurable invasive ca. Of these, 1,556 were ductal not otherwise specified (NOS), 103 were special ductal subtypes (mucinous, tubular, or medullary), and 190 were lobular ca. Univariate (T-Test and χ^2) and multivariate analyses (binary logistic regression) were performed.

RESULTS: By T stage (AJCC-1997), the frequency of ALNM was: T1mic= 3/32 (9.4%), T1a= 34/223 (15.2%), T1b= 106/501 (21.2%), T1c= 272/791 (34.4%), T2≤3cm= 126/231 (54.5%), T2>3cm= 40/58 (69%), T3= 13/13 (100%). The *p*-values for variables significantly associated with ALNM are listed in the table.

Variable	Univariate Ductal	Univariate Lobular	Multivariate Ductal	Multivariate Lobular
Age	<0.0005	<0.0005	0.003	NS
Size	<0.0005	<0.0005	<0.0005	<0.0005
Palpability	<0.0005	0.001	NS	NS
Lymphovascular Invasion (LVI)	<0.0005	0.035	<0.0005	NS
Histologic Grade	<0.0005	NA	NS	NA
Nuclear Grade	<0.0005	NA	0.036	NA
Ductal NOS vs. Special Subtype	<0.0005	NA	0.001	NA
UIQ vs. Other Quadrant	<0.0005	NS	<0.0005	NS
Multifocality	0.001	0.001	0.027	<0.0005

Table Notes: NA=not applicable, NS=non-significant.

CONCLUSIONS: Tumor size, LVI, age, nuclear grade, histologic subtype, multifocality, and location in the breast were independent predictive factors for ALNM for ductal ca. For upper-inner quadrant (UIQ) ductal ca, ALNM were less frequent than for other quadrant tumors. Only tumor size and multifocality were independent predictive factors for ALNM in lobular ca.

106 RT-PCR Analysis for Mammaglobin and Carcinoembryonic Antigen Detects Metastases in Histology-Negative Sentinel Lymph Nodes.

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Axillary staging is critical for predicting breast cancer recurrence and for guiding treatment, yet lymph node (LN) metastases are missed in many patients by current methods. To improve staging accuracy, we have combined sentinel lymphadenectomy with the sensitive technique of reverse transcriptase-polymerase chain reaction (RT-PCR). As the first investigators to identify mammaglobin (MG) and carcinoembryonic antigen (CEA) as specific dual markers for the RT-PCR detection of SLN metastases, our objective was to determine if this molecular analysis does in fact detect occult metastases. Here we present analysis of 154 SLN from 87 patients enrolled in a multi-center trial: 37% node-positive by histology (Histo+ = H&E ± IHC) and 63% node-negative. As single markers, PCR for MG and CEA identified 94% and 84% of patients with Histo+ LN. Only one patient with histologically involved LN failed to express either marker (3% false negative rate) and 78% had LN that expressed both markers. Each marker increased the detection of metastases in Histo- patients (n = 55):

MG+	CEA+	MG+ CEA+	MG+ or CEA+	MG- CEA-
15 pts (27%)	14 pts (25%)	5 pts (9%)	24 pts (44%)	31 pts (56%)

Thus, PCR detection of MG or CEA in SLN would upstage 44% of Histo- women, consistent with clinical recurrence rates. Marker expression correlates with tumor size and estrogen receptor negativity. Clinical recurrence wil l determine the significance of molecular staging: no node-negative women have recurred to date (mean follow-up 31 months). These results show that MG and CEA remain promising dual clinical markers for RT-PCR detection of SLN micrometastases for improved staging and management of breast cancer patients.

107 Variations of Success Rates for Sentinel Node Mapping in Breast Cancer: Results of a German Multicenter Study.

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Objective: Promising results have been reported for sentinel node mapping in breast cancer and numerous institutions have already abandoned routine axillary dissection for small breast cancers. The success rates reported in literature, however, vary and many questions concerning the technical proceeding are still not standardized. In order to analyze whether sentinel node biopsy was a reliable procedure, we performed a multicenter validation study and evaluated the detection rate and the sensitivity of each participating center as well as the results of different surgeons.

Material and Methods: The data of 709 patients were prospectively collected. Only patients with unicentric disease and clinically negative axillary status were included. All patients received full axillary dissection after sentinel node biopsy had been performed. The surgeon was free to chose the lymphography, as well as the injection technique.

Results: 16 centers participated in the trial and performed 709 sentinel node biopsies. The blue dye technique was used in 139 patients, lymphoscintigraphy in 127 and a combined procedure in 442 cases. The overall detection rate was 84.3% and the sensitivity 91.3%. An interinstitutional variation of success rates for sentinel node identification was found between 57.1% (Tc only and combined) and 94.1% (Tc only), the false negative rate varied between 22.2% (Tc only and combined) and 0% (all techniques). The center with the lowest detection rate had also the highest rate of false negative findings. When the data of all surgeons with less than 10 procedures were summarized, an overall detection rate of 75.4% and a sensitivity of 91.4% was found. For surgeons with more than 20 procedures the detection rate varied between 71.4% and 100% and the sensitivity between 71.4% and 100%.

Conclusion: Sentinel node mapping seems a promising tool for axillary management in breast cancer. Success rates, however, vary strongly between different institutions and surgeons. In order to obtain reliable results for the procedure, a standardized and detailed protocol for the technical proceeding is necessary, as well a defined training program.

108 A New Score for the Assessment of Postsurgery Morbidity Following Axillary Surgery.

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Introduction: One endpoint of numerous trials is the assessment of arm symptoms following new techniques of axillary surgery. Shoulder arm morbidity, however, is a complicated polysymptomatic disease and diverging techniques are currently used to assess subjective and objective symptoms. In order to optimize the assessment of this complex syndrome, we developed a specific score that summarizes the occurrence as well as the intensity of subjective and objective symptoms.

Material and Methods: 396 patients who had undergone conventional axillary surgery were thoroughly examined for postsurgical morbidity. Objective and subjective symptom occurrence and symptom intensity were evaluated using 5-point scales. Reproducible techniques were applied for clinical evaluation. According to their significance and their degree of intensity, different symptoms were summarized on a scale where subjectively and objectively assessed symptoms were each given 50% consideration. A maximum of 5 pts was assigned if no sensitivity problem was registered, 25 pts for absence of pain and 20 pts for unimpaired strength, mobility and edema respectively. According to the intensity of symptoms within the 5-point scales, score points were subtracted. A patient with 100 score points was regarded as completely free of disease.

Results: When all clinical measurements and the results from a 5-point-scaled questionnaire were summarized in a specific score, only 12 patients achieved 100 score points. 82 patients had > 90 pts, 80 women achieved between 80 and 90 pts, 73 between 70 and 80 pts, 73 between 60 and 70 pts, 34 between 50 and 60 pts and 27 had <50 pts. The median for all patients was of 77.5 score points. For different surgeons a variation of the median between 70.8 and 82.3 was registered

Discussion: Shoulder arm morbidity following axillary surgery is a polysymptomatic disease that can bes t be expressed on a score that summarizes the occurrence as well as the intensity of several symptoms. The use of a specific score facilitates the assessment of postsurgical outcome so that the comparability of clinical trials may be improved. The effect of rehabilitation measures can equally be verified.

109 Long Term Results of Randomised Studies of Axillary Clearance Vs Non-Targeted Axillary Sampling.

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Data from randomised sentinel node studies will not be available for at least a decade. 855 women with operable T_1-T_2, N_0 N_1, M_0 breast cancer were enrolled in 2 consecutive studies of mastectomy (Mx), n=401 or wide local excision (WLE) n=454 and were randomised to 4 node sampling (NS) or clearance (NCl). All patients with involved nodes on NS had axillary radiotherapy (XRT). Systemic therapy was based on node status and identical in both randomised groups. Mean follow up was 4429 days for Mx, 2538 days for WLE and 3434 days for the combined group. In the Mx and WLE groups there was no significant difference in axillary recurrence within node positive (+ve) or node negative (-ve) groups whether patients had NS or NCl. A combined updated analysis to 1999 by treatment received showed significant differences (Table).

Combined		Axillary Recurrence				Survival			
Group	n	5y	10y	15y	p value	5y	10y	15y	p value
NS -ve	283	3.7	7.2	7.2	0.023	89.8	83.9	69.5	0.36
NCl -ve	260	2.0	2.0	2.0		88.8	77.4	66.9	
NS +ve	148	7.3	10.9	10.9	0.036	75.3	58.1	49.2	0.57
NCl +ve	164	3.1	6.5	6.5		76.8	63.1	51.7	

Non-targeted axillary node sampling accurately assesses axillary status in over 95% of patients. There is a significantly higher axillary recurrence rate in the negative axilla following sampling. Survival is the same whether patients had NS or NCl. Patients with recurrence after NS were salvageable by NCl. Clearance produces significantly better control of the involved axilla than NS followed by XRT. Non-targeted 4 node axillary sampling is accurate and safe; if sentinel node biopsy improves accuracy further our data show it can be introduced before results of long term studies become available.

110 The Clinical Significance of Microinvasive Breast Cancer.

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Breast carcinomas with a focus or foci of invasion are referred to as microinvasive carcinomas. The reported frequency of lymph node metastases varies from 0 to 14%, and the survival of women with microinvasive breast cancer is not well characterized. This study was performed to study microinvasive breast cancer with the inclusion of subgroup analyses.

A review of data from the Surveillance, Epidemiology, and End Results Program of the National Cancer Institute was performed using cases diagnosed from January 1988 through December 1994. Among women with microinvasive breast carcinoma undergoing both a resection of the primary malignancy and an axillary lymph node dissection there were 822 cases without and 104 cases with lymph node metastases. Survival differences were calculated using actuarial survival rates.

Among women with microinvasive breast cancer the frequency of lymph node metastases was 11.2%. The frequency of lymph node metastases varied with grade (P=0.009), and histologic diagnosis (P=0.003), but not with age (P=0.23). Cases with lymph node metastases experienced a survival disadvantage at eight years when compared to cases without lymph node metastases (66.9% vs 88.0%, P=0.0001). In subgroup analyses, cases with lymph node metastases experienced a survival disadvantage when compared to cases without lymph node metastases as a function of grade (grade 2, P=0.035; grade 3, P=0.0001; unknown grade, P=0.006), histology (infiltrating ductal carcinoma, P=0.0001), and age (<50, P=0.0001; ≥50, P=0.0003). Among women with microinvasive breast cancer the frequency of lymph node metastases is sufficient to warrant examination of the axillary lymph nodes. Lymph node metastases when present are associated with a survival disadvantage in most, but not all subgroups examined.

111 Intraoperative Assessment of the Sentinel Node in Breast Cancer: Comparison of Frozen Sections Using Immunohistochemistry and H+E Sections with Traditional Postoperative Assessment.

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INTRODUCTION: Postoperative histopathological assessment of the sentinel node using conventional paraffin haematoxylin and eosin (H+E) sections takes several days. Therefore, patients with a positive sentinel node would require delayed axillary treatment. Intraoperative assessment of the sentinel node using frozen sections can be performed during surgery to the primary cancer, and allows immediate axillary clearance when necessary. Frozen sections may be assessed using H+E staining or by immunohistochemistry (IHC). Cytokeratin 19 (CK 19) is reliably expressed by breast cancer cells and is a suitable target for IHC.

METHOD: 36 sentinel nodes were bisected. One half was processed routinely for paraffin H+E sections. Frozen sections were cut from the remainder and both H+E staining and IHC performed to detect metastasis. The Enhanced Polymer One-step Staining (EPOS) antibody to CK 19 (DAKO) was used, as it allows particularly rapid immunostaining. The sections were assessed by a consultant breast pathologist and the results documented for later comparison with the paraffin H+E sections. The technique requires less than 30 minutes.

RESULTS: H+E paraffin section demonstrated 13 positive and 23 negative sentinel nodes. EPOS frozen section detected metastasis in 10 out of the 13 positive nodes, as did H+E frozen section. There were 3 false negative nodes using both techniques. The sensitivity of both EPOS and H+E frozen sections was 77% and the false negative rate was 23%. However, there was much greater contrast between cancer cells and the background using the EPOS technique. EPOS and H+E frozen sections correctly assessed all 23 negative sentinel nodes. Therefore, the specificity was 100% and the false positive rate was 0%.

CONCLUSIONS: Frozen sections allow rapid assessment of the sentinel node. The false negative results could be due to sampling error, as metastasis may have been present in the other halves of the nodes only. The excellent contrast provided by EPOS IHC indicates the potential for improved accuracy over H+E frozen section, which may be detected by further study.

112 Effect of Blue Dye on Pulse Oximetry during Sentinel Node Biopsy.

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INTRODUCTION: During sentinel node biopsy for breast cancer, patients have developed blue discolouration of mucous membranes, with a concurrent fall in measured oxygen saturation. This has caused concern that the patient is cyanotic. The aim of this study was to determine whether the peritumoural injection of blue dye used to localise the node causes a reduction in the pulse oximetry reading.

METHOD: A case-control study of 120 patients undergoing primary breast cancer surgery was performed. Pulse oximetry measurements were recorded for 60 cases undergoing sentinel node biopsy and 60 age-matched controls who had conventional axillary surgery. Oximetry readings were recorded at 30 minute intervals during the operation and at 15 minutes during recovery.

RESULTS:

Time after induction (Min)	Difference in mean saturation (%)	Time in recovery (Min)	Difference in mean saturation (%)
0	0.27	0	1.17
30	0.25	15	0.65
60	0.99	30	0.59
90	0.88	45	0.96
120	1.00	60	0.67
150	1.02		

There was a significant difference between the pulse oximeter readings of the cases and controls from 60 minutes after induction until 60 minutes postoperatively ($p<0.05$).

CONCLUSIONS: Patent blue V causes a reduction in pulse oximeter measurement of oxygen saturation after absorption into the circulation, and the anaesthetist should be warned of this prior to its injection. Further study measuring oxygen saturation by arterial blood gases would confirm whether this is an artefact.

113 The Intradermal Sentinel Node: Update 2000.

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As sentinel node biopsy gains widespread use in the surgical community, a growing number of techniques have been developed to aid in rapid and successful identification of this node. Each of these new techniques, however, must match the accuracy data in older and larger series, so as to limit the risk of understaging.

This procedure, initiated about the same time as the major groundbreaking series from Tampa and Santa Monica, and updated for the third consecutive year exclusively at this meeting, provides such data. The series was initiated in 6/95 and is now updated with 325 patients in whom an intradermal injection of radioisotope was the only method used to retrieve the axillary sentinel node. As before, the sentinel node is identified 100% of the time in a patient population of average age 74, with average tumor size 1.4 cm. The node is identified in approximately 30% of patients with invasive cancer but 0% in cases of DCIS, with two false negatives. One "recurrence" has been documented in the axilla thus far, to be described. The percent-sentinel node positivity vs. tumor size will be provided in both 5 mm. increments of tumor size and with each T-stage. This data matches that in other reported series and has been constant in the three years I have been updating this series.

As this and other series, employing different injection techniques grow and mature, it is clear that the accuracy of sentinel node retrieval and staging is not compromised. This data lends support to the growing consensus that a single axillary sentinel node drains the entire breast and that it can be identified with different injection techniques.

114 Predictive Value of the Sentinel Lymph Node in Breast Cancer: How Many Sentinel Nodes Are Enough?

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Introduction: Sentinel lymph node biopsy (SNB) has been shown to be an accurate, less invasive means of determining nodal status in breast cancer. Most patients manifest with multiple sentinel nodes, which require potentially more expense, morbidity and resources to harvest and evaluate. The purpose of this study is to determine the minimal number of sentinel nodes necessary to determine the pathologic status of the axillary basin in breast cancer.

Methods: 352 consecutive breast cancer patients undergoing SNB were reviewed to determine the predictive value of sentinel nodes harvested. Sentinel nodes were defined as either blue, hot (> 2x background) or both. Sentinel nodes were ranked (1-4+) at the time of surgery based on the order in which they were harvested.

Results: Sentinel lymph nodes were successfully identified in 312 (89%) patients. Of these patients, 80 (26%) had pathologically involved nodes (H+E or IHC+) mandating completion axillary dissection. An average of 1.95 (range 1-16) sentinel nodes were harvested per patient, 39 patients had 2 or more, 24 with 3 or more and 12 with 4 or more sentinel nodes. SN #1 independently predicted the pathologic status of the axilla in 71 (92%), SN #2 in 6 (7%, positive SN #2 was "hotter" than negative SN #1 in 5 of 6 patients) and SN #3 in 1 patient. All positive SNs were blue.

Conclusions: The pathologic status of the axilla is independently determined by the first or second SN harvested in 99% of patients. The single "hottest" SN determines axillary involvement in 99% of patients. These findings may: improve patient care by limiting dissection; improve predictive value by allowing for focused pathologic evaluation of the first, or hottest nodes harvested; and potentially facilitate therapeutic sentinel lymphadenectomy in select patients.

115 Effect of Technetium 99m Sulphur Colloid Injection Interval (TII) on Sentinel Node Biopsy (SNB).

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Introduction: SNB alone without complete lymph node dissection is becoming the standard of care for breast cancer patients treated by experienced surgeons. The best time interval between radioisotope injection and SNB dissection (TII) has not been determined. It has been hypothesized that a short TII will decrease the chance of finding a sentinel node and a long TII will allow labeling of non-sentinel nodes. Objective: To determine the optimal TII for breast cancer SNB. Methods: An IRB approved multi-center clinical trial enrolled breast cancer patients between 1996 and 2000 for SNB using a peri-tumor injection of technetium 99m sulfur colloid and isosulfan blue dye. Eighteen academic and private practices participated and the TII was not restricted. The SN was defined as either blue only, both blue and hot or hot only. Results: Of 648 breast cancer patients enrolled, 544 had data to calculate the TII. The majority of patients had a complete lymph node dissection (562, 87%) and only this group was used to calculate the false negative rate. The false negative rate for the entire group was 11.9% , with 167 patients found to have metastatic disease in their axilla. The table summarizes the false negative and identification rates found in specific TII intervals. The TII >60<300 had a higher identification rate than <60 minutes (p=0.0021). The false negative rate tended to be higher for TIIs <20 and >300 minutes.

Conclusion: The optimal TII for breast SNB is probably between one and five hours. A larger group of false negatives would need to be analyzed to determine if timing influences the false negative rate.

TII (min.)	≤20	>20≤60	>60≤180	>180≤300	>300
N	80	69	178	151	66
Identification rate	83%	83%	92%	93%	85%
False negative rate	18%	6%	10%	9%	14%
Mean # SNs	1.89	1.92	2.28	2.45	2.34

116 Predictors of Nonsentinel Node Metastasis in Breast Cancer Patients: Size and Extracapsular Extension of the Sentinel Node Metastasis.

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BACKGROUND: For many breast cancer patients who undergo intraoperative lymphatic mapping and sentinel lymphadenectomy (LM/SL), the sentinel node (SN) is the only tumor-involved node. Investigators have sought predictors of nonsentinel node (NSN) metastasis in patients with a SN metastasis. In our initial institutional SN validation trial, extracapsular extension (ECE) of the SN metastasis was associated with NSN metastasis on multivariate analysis. We hypothesize that with our expanded LM/SL experience, ECE of the SN metastasis will prove to be highly predictive of NSN tumor involvement.

METHODS: Between April 1998 and April 2000, 182 patients with clinical T1 or T2 N0 M0 breast cancer underwent attempted LM/SL at the University of North Carolina. LM/SL was performed using a blue dye and technetium sulfur colloid technique. In all cases with a tumor-involved SN, an axillary lymph node dissection (ALND) was recommended. ECE was defined by tumor spread through the capsule of the SN. The size of the metastasis was measured by a dedicated breast pathologist using an ocular micrometer. Statistical analysis, with Pearson chi-square tests and multiple logistic regression, was performed.

RESULTS: The SN was successfully located in 173 (95.1%) cases and contained tumor in 51 (29.5%) cases. ALND was performed in 49 of the 51 cases; two patients refused the recommended surgery. ECE of the SN metastasis was present in 15 (30.6%) cases. Patients with ECE of the SN metastasis were more likely to have NSN tumor involvement (80% vs. 32.4%, p=0.002) and a greater total number of tumor-involved nodes (6.5 vs. 1.9, p=0.001) than patients without ECE of the SN metastasis. Increasing size of the SN metastasis, examined as a continuous variable, was also associated with an increased likelihood of NSN tumor involvement (p=0.001).

CONCLUSION: ECE and increasing size of the SN metastasis are highly predictive of NSN metastasis. ALND should be strongly recommended for all breast cancer patients whose SN metastasis is >5.0mm or demonstrates ECE.

117 Patterns of Internal Mammary Drainage during Sentinel Node Lymphoscintigraphy.

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Postmatectomy radiation has recently been shown to improve overall survival for some node positive patients. Whether internal mammary (IM) radiation contributes to the clinical benefits is controversial. Individual physician recommendations vary from exclusion of the IM nodes, inclusion of all IM nodes, to modified tangential treatment encompassing the upper IM nodes. The purpose of this study was to examine whether sentinel node lymphoscintigraphy may help identify patterns of IM node drainage for purposes of radiation planning.

Sentinel node lymphoscintigraphy was performed using four peritumoral injections of 1 ml technetium sulfur colloid directed by physical examination or ultrasound guidance. Eighty-nine studies performed during the period of 1997-1998 were reviewed for the presence of IM drainage. Sixteen studies (18% of the total) were located which demonstrated IM drainage, with or without associated axillary sentinel nodes. These studies and associated operative and pathologic findings were reviewed in detail. The number and location of sentinel nodes was assessed. IM location was considered "upper" if it occurred at or above the third rib, and "lower" if it was inferior to that point.

Of the 16 patients with IM drainage, 7 had IM drainage only and 9 had additional axillary drainage. Eight studies identified only one IM node, and eight identified more than one. The average number of IM sentinel nodes identified in these patients was 1.9; the average number of axillary nodes identified was 1.1. Ten patients (63%) had pathologic axillary node metastasis. Of inner/central tumors with IM drainage, 66% were axillary node-negative as compared to 20% of outer quadrant tumors with IM drainage. For all patients, 18 of 30 (60%) visualized IM nodes were in the lower IM region. Among those with only IM drainage, 5 of 11 (45%) were in the lower region. Where there was only a single IM sentinel node, 2 of 5 (40%) were in the lower region. For patients with pathologic axillary metastasis, 12 of 20 (60%) IM sentinel nodes were in the lower region. For lower quadrant breast tumors, 86% of IM sentinel nodes were in the lower IM region.

Sentinel node lymphoscintigraphy may identify patterns of IM drainage. Approximately half of all IM sentinel nodes in this experience were located in the lower IM region, with potential implications for radiation planning.

118 Prognostic Effects of Circulating Insulin-Like Growth Factor Binding Proteins (IGFBP's) 1 and 3 in Operable Breast Cancer (BC).

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The IGF family of growth factors is believed to play a role in the development and progression of BC, however, clear evidence of important prognostic effects is lacking. We have recently identified a strong adverse prognostic effect of circulating insulin but not IGF's I and II in operable BC; we now report effects of circulating IGFBP's 1 and 3.

512 women underwent mastectomy or lumpectomy with axillary node dissection for T1-3, N0-1, M0 BC. Fasting blood was collected and weight/height measured. Blood was analysed for IGFBP's 1 and 3. Information on traditional prognostic factors and treatment was recorded; women were followed for distant recurrence and death. Mean age was 50.4±9.7 years. 288 were T1, 164 T2, 24 T3 and 36 TX. 356 were N0. 314 were ER positive, 285 PgR positive. 197 were postmenopausal. 147 received adjuvant chemotherapy only, 151 tamoxifen only, 46 both and 168 neither. IGFBP-1 levels were inversely correlated with insulin (Spearman $r = -0.60$, $p < 0.0001$), reflecting known inhibition of IGFBP-1 gene expression by insulin. In univariate analysis, low levels of IGFBP-1 significantly predicted distant recurrence and death, RR (95% CI) for lower versus upper quartile 2.8 (1.3-6.1) and 4.7 (1.7-13.1) respectively. These effects persisted after adjustment for tumor stage and grade, nodal stage, ER, PgR and adjuvant therapy. They did not persist after adjustment for insulin alone or in Cox models containing insulin, other prognostic and treatment variables ($p > 0.20$). High levels of IGFBP-3 predicted distant recurrence (RR upper versus lower quartile 3.1, 95% CI 1.2-7.8) but not death (RR 0.9, 95% CI 0.3-3.2). The effect on distant recurrence was restricted to postmenopausal women (RR 12.1, 95% CI 2.5-57.9) and it persisted after adjustment for nodal and tumor stage, tumor grade, ER, PgR and adjuvant therapy in these women.

Prognostic effects of IGFBP-1 appear related to the known effect of insulin on IGFBP-1 gene expression. The adverse effect of IGFBP-3 on distant recurrence in postmenopausal women requires replication. Paracrine secretion, which may contribute prognostic effects, has not been examined.

119 Urokinase Plasminogen Activator (uPA) and Its Type-1 Inhibitor (PAI-1) Are Superior to the Nottingham Prognostic Index NPI in Predicting Relapse in Node-Negative Breast Cancer Patients.

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About 70 percent of the node negative breast cancer patients can be cured by surgery and radiation alone. A general recommendation for adjuvant chemotherapy is questionable, in particular, when increasing side effects and rising costs due to new chemotherapeutic drugs are taken into account.

The urokinase plasminogen aktivator (uPA) and its type-1 inhibitor (PAI-1) play an importent role in invasion and metastasis of solid tumors. In the present prospective study we investigated the prognostic value of determining the proteolytic capacity of breast cancer tumors in comparisson to the Nottingham prognostic index (NPI). Urokinase plasminogen activator and its type-1 inhibitor were detected by an enzyme-linked immunosorbent assay, in tissue extracts from 235 patients with primary breast cancer, treated at our hospital. The NPI is based on lymph-node stage, tumor size and pathological grade. Median duration of follow-up was 60 months. The Cox proportional hazards model was applied for both univariate and multivariate analysis. The NPI ($p<0.0001$) and lymph node status ($p<0.0001$) were found to be strongly positively related to relapse-free survival, followed by PAI-1 ($p=0.0005$), grading ($p=0.0006$) and uPA ($p=0.0010$). In multivariate analysis only NPI ($p<0.0001$) and PAI-1 ($p=0.0008$) were positively associated with relapse-free survival.

Univariate subgroup analysis of 126 node-negative cases showed that uPA ($p=0.003$), PAI-1 ($p=0.005$) and grading ($p=0.009$) were strong predictors of relapse-free survival, while the NPI was not significantly associated with the rate of relapse. In the multivariate analysis only uPA ($p=0.0047$) remained as an independent prognostic factor for relapse-free survival.

The NPI has proved to be a strong and independent predictor of relapse in breast cancer patients in general, but lack prognostic power in the clinically highly important node-negative subgroup. Proteases as uPA and its inhibitor PAI-1 are independent markers for relapse, thus enabling oncologists to chose new and more effective therapies for those patients who are at risk and prevent the low risk breast cancer patients from overtreatment.

120 Diabetes but Not Obesity Is a Prognostic Factor for Disease Free Survival (DFS) in Women with Stage I, II, or III Breast Carcinoma Receiving Tamoxifen.

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Obesity has been found to be a poor prognostic factor of breast cancer in previous studies. Diabetes is associated with obesity. This study examined the association between breast cancer DFS, obesity and diabetes. Women newly diagnosed at Harper Hospital with stage I, II, or III breast cancer between 1/90 and 12/96 were included for DFS analysis. Variables were derived from SEER database, KCI clinics, hospital and individual physicians' office records, and 1990 census data including tumor characteristics, treatment and concurrent medical conditions, socio-demographic information, and recurrence and survival information. Among 1126 women, 625 were African-American and 501 were Caucasian, median age 58 years. Stage I was 41% and stage II was 47%. Estrogen receptor (ER) positive was 60% and progesterone receptor (PR) positive was 53%. 357 patients received tamoxifen treatment only, 247 received adjuvant chemotherapy only, 179 patients had both, and 207 no systemic treatment. 347 were overweight (BMI 25-29.9) and 362 were obese (BMI >=30). Of 159 diabetic patients, 62% were overweight or obese. In univariate analysis, the relative risk (RR) of recurrence associated with obesity was 1.2 (95% CI 0.9-1.5) and with diabetes 1.5 (95% CI 1.0-2.0). In multivariate analyses, diabetes remained a significant prognostic factor after controlling for age, tumor size, and positive lymph node status (RR 1.4, 95% CI 1.0-2.0). After adding ER and PR status, diabetes was not a statistically significant factor. When analyses were restricted to the 551 patients who received tamoxifen treatment, the RR of recurrence associated with diabetes was 2.2 (95% CI 1.3-3.8) indicating relative tamoxifen resistance. After controlling for age, tumor size, positive lymph node status, tumor differentiation, and ER and PR status, diabetes but not obesity, at the time of diagnosis was a significant prognostic factor. The effect of diabetes on DFS was independent of obesity. The absence of an obesity effect might be due to advances in adjuvant breast cancer treatment introduced in the last 15 years. The persistence of diabetes as a prognostic factor independent of obesity and tumor characteristics has therapeutic and biologic implication.

121 Elevated Cytosol Vascular Endothelial Growth Factor (VEGF) Showed a Significant Influence on Outcome: Retrospective Analysis of 173 Patients with Stage I and II Breast Cancer.

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Introduction: Angiogenesis is regulated by the balance of molecules that stimulate angiogenesis, such as vascular endothelial growth factor (VEGF), and negative regulators such as angiostatin and endostatin. Elevated VEGF serum levels are associated with poorer outcome. Different methods have been described to measure VEGF either in serum or in tumour tissue. Our main objective in the present study is to determine relapse free survival (DFS) and overall survival (OAS) related to elevated VEGF levels in cytosols of primary breast cancer cells.

Material & Methods: Breast cancer samples have been frozen and stored in liquid nitrogen. The samples were homogenised by ultra-turrax. Retrospectively, Cytosol levels of VEGF were measured using enzyme - linked immunosorbent assay (Quantikine, R&D systems, Oxon, UK).

The follow up of these patients (pts) was updated and DFS and OAS was estimated by Kaplan-Meier-Method. Cox model was used for multivariate analysis.

Results: We analyzed VEGF in the cytosol of 173 patients with stage I or II.116/173 samples were from invasive ductal carcinomas, 57/173 from lobular carcinomas. The median age of pts was 56 y, 80/173 pts were lymph node negative. Median follow up is 28 months. VEGF levels were significantly lower in lobular carcinoma specimens compared to ductal carcinomas. In invasive lobular carcinomas the median value was 57pg/mg and in ductal carcinomas it was 266pg/mg total protein. VEGF values higher than the median were regarded positive. Using univariate analysis there was a significant difference in relapse free survival for all pts ($p = 0.02$). This could be confirmed esp. for invasive lobular carcinomas ($p = 0.009$). Using multivariate analysis elevated VEGF cytosolic levels showed no prognostic value compared to nodal status and grading, $p = 0.03$ and $p = 0.09$ respectively.

Conclusion: Elevated VEGF levels, measured in serum, tissue or cytosol, are associated with aggressive growth of cancer. Differences in analysing VEGF are still common and not comparable. The here described cytosol VEGF evaluation is reliable and shows a strong but not independent correlation to patient outcome. The median value as cut-off definition might not be ideal and has to be correlated to the pts follow up. Our data strongly support VEGF as target molecule in breast cancer.

122 Age-Dependent Breast Cancer Prognostic Markers.

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The expected increase in breast cancer development associated with an aging population will occur largely in ≥40y old women in which worldwide incidence rates are most variable and chemoprevention strategies most promising. To identify markers linked to age-dependent breast cancer biology, we analyzed tumor parameters previously measured for two separate primary breast tumor collectives (n=850 paraffin-archived, n=3000 cryobanked primary tumors), each with a median 61y patient age at diagnosis. These analyses confirm that tumors diagnosed at <40y age are associated with markers of more rapid tumor growth (increased S-phase, mitotic index, Ki67) and perhaps greater genetic instability (nuclear grade, abnormal p53). In contrast, measures of tumor invasiveness including degree of nodal involvement and protease production (cathepsin D, uPA) appear to be age-independent. Overexpression of the membrane and nuclear receptors, ErbB2 and ER, show a significant and reciprocal age relationship with one another; however, despite the higher frequency of ER overexpression and median level of tumor ER content with each increasing age decade there is a significantly reduced correlation between ER-positivity and co-expression of the ER-inducible genes, PR and pS2. Of interest, normal breast tissue with its 10-fold lower ER content compared to adjacent breast tumor shows a comparable age-dependent increase in ER expression. Preliminary evidence also suggests an age-dependent relationship for markers responsive to oxidant stress (increased Erk5 signaling, loss of Sp1 DNA binding). Multivariate survival analysis of age-dependent tumor markers is pending to identify those that may be biologically linked to breast cancer development in ≥40y old women.

123 Expression of Estrogen Receptor β Protein in Human Breast Cancer: Correlation with Clinical Tumor Parameters.

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The recent discovery of a second estrogen receptor, designated ERβ, raises pressing questions about its role in estrogen regulation of human breast cancer cells, and its significance for the prediction of recurrence and treatment responses in clinical breast cancer. Most of what we know about ERβ expression comes from studies examining a limited number of samples at the RNA level. We have generated a monoclonal antibody useful for the detection of ERβ protein in archival, formalin-fixed breast tumors, and have examined its level using immunohistochemistry (IHC) in a pilot series of 242 breast tumor specimens from the Baylor SPORE Tumor Bank. Co-expression of ERβ and ERα was found in the majority of the tumors, with 76% of the tumors expressing ERβ. ERα, but not ERβ, was strongly associated with progesterone receptor (PR) expression, suggesting that ERα is the predominant regulator of this estrogen-induced gene in breast tumors. Although ERα expression was positively correlated with low tumor grade, diploidy and low S-phase fraction, all biological parameters of a good prognostic profile, ERβ trended toward an association only with aneuploidy; no association with tumor grade or S-phase fraction was seen for ERβ. These results suggest that ERβ expression is not just a surrogate for ERα in breast tumors, and thus may be a useful prognostic marker in clinical breast cancer. Studies examining the role of ERβ as a predictor of response to antiestrogen treatment in a larger series of patients are underway and will be discussed.

124 C-erbB-2 as a Prognostic and Predictive Factor in Breast Cancer (BC): A Meta-Analysis.

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Despite a large body of research, the clinical utility of erbB-2 as a prognostic or predictive factor in BC is still not clearly established. To evaluate the value of c-erbB-2 as a pure prognostic factor (i.e. assessed only in patients (pts) who did not receive adjuvant systemic therapy (AST), and as a pure predictive factor (i.e. only in pts who did receive AST), we conducted a meta-analysis. Using standard methods for literature search we identified 238 studies of c-erbB-2 in BC:

32 studies could be assessed for *prognostic relevance*, and 98 studies could be assessed for *predictive relevance* (based on ability to determine erbB-2 effect in pts without and with use of AST. The effect of erbB-2 could not be evaluated in the remaining studies because they did not clearly indicate AST use (103 studies) or did not provide erbB-2 results separately for lymph node (LN) negative (neg) and positive (pos) pts.

Prognostic Relevance: Only about half of the 32 studies provided adequate quantitative data to estimate pooled RR and 95% CI for relapse free survival (RFS) and overall survival (OS). The pooled RR (95% CI) were as follows:

RFS: 1.12 (1.04, 1.22) for LN neg pts; not evaluable for LN pos pts

OS: 1.15 (1.07, 1.23) for LN neg pts; 1.38 (1.18, 1.62) for LN pos pts

One study contributed a possibly disproportionate amount to the total weight in calculating the pooled RRs for LN neg studies. When a modified weight was substituted, the RRs for RFS and OS were 1.84 and 1.55, respectively. What is clear, however, is that a reasoned assessment of prognostic relevance of erbB2 can be based on only a minority of available studies, which demonstrate considerable variability. Even ignoring the possible bias of this selected subset, erbB-2 appears to be only a weak-to-moderate prognostic factor. Thus, the available data do not provide a sufficient basis for an informed decision on the prognostic role of erbB-2 in BC.

Predictive Relevance: Analyses are underway and will be reported at the meeting.

125 Clinical Impact of Two New Prognostic Factors (VEGF and $p27^{kip1}$) on Conventional Risk Categories for Node-Negative (N-) Breast Cancer.

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Univocal evidence has not yet been provided of the prognostic and predictive strength of biomarkers or their actual clinical impact. A growing skepticism regarding the usefulness of biomarkers has led to a progressive reduction of their use in clinical practice, and the attitude of some clinicians to administer systemic treatments to all breast cancer patients regardless of the tumor biologic profile was supported by outcome of the EBCTG meta-analyses, which demonstrated the reduction in relapse and mortality following adjuvant hormonal or cytotoxic treatments. However, a renewed interest in assessing the clinical impact of biomarkers has recently emphasized the need for guidelines for an appropriate and standardized use as well as for designing, conducting, analyzing and reporting translational studies. For N- tumors, a critical issue is the assessment of the potential benefit of refining by novel putative prognostic factors the risk categories propose by the 1998 St. Gallen Consensus Conference and based on conventional factors, such as tumor size, ER status, age, grade and/or tumor proliferative activity. By combining the variables it is possible to separate patients within subsets at a minimal risk of 5-year relapse (<10%, for tumors <1 cm, ER+, slowly proliferating, for patients >35 years), a high risk (>25%, for tumors >2 cm or ER- or rapidly proliferating, or for patients <35 years), and an intermediate risk (between 10% and 25%, for all the other cases). The strength of novel prognostic factors could be classified according to their ability to change the risk category of individual patients as recently proposed by Hayes et al. (BCRT 52:305-19, 1998). On 253 consecutive patients with N- resectable tumor submitted to surgery alone until relapse, and with a median 5-year follow-up, VEGF levels and $p27^{kip1}$ expression, were significantly associated with clinical outcome (P=0.07 and P=0.005). When analyzed within risk categoris, both proved to be moderate prognostic factors, since they moved patients across two categories, with a complementary impact. In fac, VEGF levels down-graded the risk within all the three categories (probabilily of relapse for low vs high VEGF: 0% vs 11% in the minimal, 5% vs 14% in the intermediate, and 14% vs 28% in the higk-risk subset), whereas $p27^{kip1}$ contributed to a better prognostic refinement only within the high-risk category (probability of relapse for high vs low $p27^{kip1}$, 13% vs 50%).

126 The Subcellular Localisation of Cyclin B, Cdc2 and $p21^{WAF1/CIP1}$ in Breast Cancer: Association with p53 Status and Prognosis.

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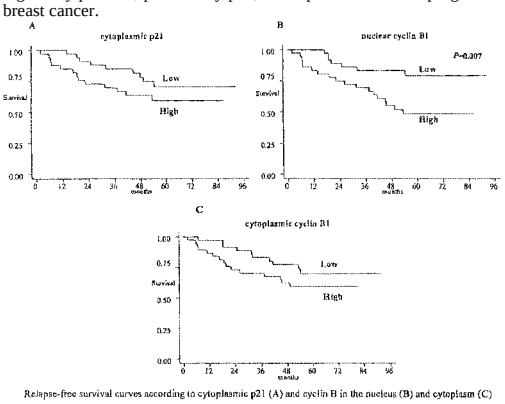
Entry into mitosis is regulated by cyclin B/Cdc2 kinase whose activity may be inhibited by the tumour suppressor p53, through its transcription of $p21^{WAF1/CIP1}$. Nuclear p21 functions as a cyclin-dependent kinase (CDK) inhibitor, in part through its ability to promote the nuclear localisation of cyclin B/Cdc2 (Oncogene 1998; 17:673-84). This study investigates the association between p53 and the subcellular distribution of cyclin B, Cdc2 and p21 in breast cancers using immunohistochemistry, and examines their relationship to prognosis.

Methods: 73 patients with unilateral, non-metastatic disease comprised 67% node-negative and 33% node-positive women. Median follow-up was 65 months. Nuclear p53 was graded from 1-3. p21, cyclin B and Cdc2 (nucleus and cytoplasm) were graded to produce an intensity distribution score.

Results: Increased cyclin B expression (nuclear and cytoplasmic) was associated with reduced OS (P=0.02) and RFS (P=0.005). Some tumours showed nuclear p21, with many demonstrating a predominant cytoplasmic p21, which was associated with cytoplasmic cyclin B (P=0.006) and increased nuclear p53 staining (P=0.03). Cytoplasmic p21 predicted reduced OS (P=0.002) and RFS (P=0.01).

Cytoplasmic p21 may have a diminished CDK inhibitory function and may be a marker for abnormal p53. We conclude that the subcellular distribution of cell cycle regulatory proteins, particularly p21, could provide valuable prognostic markers in breast cancer.



Relapse-free survival curves according to cytoplasmic p21 (A) and cyclin B in the nucleus (B) and cytoplasm (C)

127 Validation of the Prognostic Significance of Mitosin in Node-Negative Patients.

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Mitosin is a nuclear phosphoprotein that is expressed in the late G_1, S, G_2, and M phases of the cell cycle but not in G_0. We previously reported correlations between immunohistochemical (IHC) staining using mitosin monoclonal antibody 14C10 and disease-free survival in a pilot study of 386 node-negative patients (Clark et al. Cancer Res 57:5505-8, 1997). We now present results of a validation study that included a repeat analysis of 301 of the original patients, and an additional 432 node-negative patients.

Tumors were obtained fresh at the time of diagnostic biopsy or mastectomy, snap-frozen, pulverized in liquid nitrogen for steroid receptor assays, and stored at -70°C. Permanent histologic sections were prepared from the pulverized tumor, as described previously (Allred et al. J Histotechnol 16:117-20, 1993). Immunostained slides were evaluated microscopically at x400 magnification and scored for percentage of positive tumor cells.

The within-tumor Spearman correlation coefficient for mitosin scores for the repeated assays was 0.74 (p<0.0001). Using our previously defined cutpoint of 5% positive cells to define high mitosin, 78% of the repeated cases gave concordant results (Kappa=0.53, p=0.004). Mitosin scores were positively correlated with S-phase fraction by flow cytometry (r_{sp}=0.55), Ki67 by MiB1 staining (r_{sp}=0.21), and p53 by IHC (r_{sp}=0.40), and were negatively correlated with ER (r_{sp}=-0.46) and PgR (r_{sp}=-0.37). When mitosin was classified as high or low, strong associations were observed with disease-free survival of the new patients (hazard ratio=1.9, p=0.003) and the combined group of patients (hazard ratio=1.4, p=0.018). Mitosin retained its prognostic significance in multivariate analyses that included tumor size, age, ER, and PgR (hazard ratio=1.4, p=0.04). No associations were observed with overall survival.

128 The Complex between Urokinase and Its Type-1 Inhibitor in Primary Breast Cancer: Relation to Survival.

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We examined the relationship between tumor tissue level of the complex of urokinase (uPA) formed with its type-1 inhibitor (PAI-1) and survival of breast cancer patients. The study included 342 axillary lymph node-negative and -positive primary breast cancer patients with a median follow-up of 67 months. Using a newly established ELISA, the levels of preformed uPA:PAI-1 complex were measured in tumor tissue extracts and analyzed with respect to total uPA, total PAI-1, and clinicopathological parameters, including survival.

uPA:PAI-1 complex comprised a minor, variable fraction of both total uPA and PAI-1 levels. The complex levels were higher in node-negative tumors than in node-positive tumors, and higher in small and low grade tumors (all, P<0.003). The tumor levels of complex, uPA and PAI-1 were all associated with survival; high complex levels predicted longer recurrence-free (P=0.03) and overall survival (P=0.005), whereas high uPA or PAI-1 levels significantly predicted shorter survival. In multivariate Cox analysis, the only parameters that independently predicted survival were PAI-1 level and lymph node status for recurrence-free and overall survival, and additionally steroid hormone receptor status and grade for overall survival.

This is the first demonstration of a relationship between uPA:PAI-1 complex tumor level and patient survival. However, total PAI-1 level shbowed superior prognostic power. Further studies are needed to understand the relationship of these parameters to the cancer biology and to assess the clinical utility of uPA:PAI-1 complex.

129 Tissue Arrays for the Molecular Profiling of Inflammatory Breast Cancers (IBC).

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Breast cancer is a diverse clinical disease, and this diversity is driven by multiple genetic alterations and molecular heterogeneity. Inflammatory breast cancer (InflBC) with its poor prognosis and distinct clinical manifestations is illustrative of this diversity. However, little is known about the distinguishing biology and molecular phenotype that contributes to its aggressive behavior. To better understand the biology of InflBC, the protein expression profile of a range of molecules was determined on 75 clinically defined InflBC and compared with 2,093 infiltrating breast cancers of no special type (NST). Tissue arrays were first constructed by harvesting 5x3 mm cylinders containing approximately 100 mg of tumor tissue from a paraffin block of formalin-fixed, frozen tumor powder. Cylinders were re-imbedded 12 per array in paraffin, thin sections were cut, and molecular marker analysis by immunohistochemistry was performed. Consistent with their fulminant clinical course, nearly all InflBC had a high proliferative fraction (Ki67) 93% compared with 11% of NST. Although InflBC were more likely to be ER- and PgR-negative (49% and 68% of InflBC compared with 30% and 43% of NST), approximately half of InflBCs remained hormone receptor-positive. Surprisingly, erbB-2 overexpression and p53 accumulation were found less often in InflBC than NST (17% vs. 26% and 32% vs. 52%, respectively), and the proapoptotic BAX expression was markedly increased in InflBC than NST (98% vs. 67%). EGFR and bcl-2 expression was similar in the two types. Cell adhesion molecules CD44 and E-cadherin were expressed in 67% and 87% of InflBC, respectively.

Conclusion Using small amounts of material, tissue arrays can be used to assess multiple biomarkers in breast cancer. Mirroring their distinct clinical behavior, InflBCs have a molecular profile that is substantially different from NST tumors, and this potentially could be used to guide management decisions in the future. We are continuing to further refine this profile and investigate its implications for the biology and treatment of InflBC.

130 Prognostic Significance of a Novel Hypoxia Regulated Marker, Carbonic Anhydrase IX (MN/CA IX), in Invasive Breast Cancer.

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MN/CA IX is a member of the carbonic anhydrase family, with its expression found primarily in neoplastic cells and not the normal surrounding tissue (except within the GI tract). We have recently shown that MN/CA IX expression is significantly increased in hypoxic conditions across various cell lines. In this study we sought to assess the frequency of expression and the prognostic significance of this novel biomarker in a cohort of patients with invasive breast cancer. MN/CA IX expression was evaluated by immunohistochemistry with a murine monoclonal antibody, M75, in a series of 103 women treated surgically for early breast cancer. The majority of patients received adjuvant hormonal or chemotherapy. The frequency of expression and its association with other standard prognostic factors was evaluated. Univariate and multivariate analyses were performed to investigate if MN/CA IX expression independently correlated with a worse outcome. MN/CA IX expression was found in 49/103 (49.5%) of this cohort of patients. Its expression was found to be significantly associated with a higher tumor grade ($p=0.003$), a negative estrogen receptor status ($p<0.001$) and tumor necrosis ($p<0.001$). By log-rank test MN/CA IX expression was associated with a significantly worse relapse-free survival ($p=0.004$) and a worse overall survival ($p=0.001$). By multivariate analysis the hazard ratios associated with MN/CA IX expression and relapse-free and overall survival were 2.13 (95% C.I. 0.96 - 4.69) and 2.61 (95% C.I. 1.01 - 6.75) respectively. MN/CA IX expression in this cohort of invasive breast cancers was found to be a new and independent prognostic factor. Expression of MN/CA IX was associated with a worse relapse-free and overall survival. Further work assessing its prognostic significance in breast cancer and other neoplasms are warranted.

131 Data from the SEER (Surveillance, Epidemiology, and End Results) DataBase Demonstrates the Validity of Using Routine Histologic Grading for Prognostic Assessment of Patients with Early Breast Cancer.

Khan Q, Ravdin PM.

There has been a debate as to the value of histologic grading in defining the prognosis of node negative breast cancer patients. This debate has largely been drive by the question of whether the average pathologist could produce estimates of histologic grade of patients with breast cancer which had prognostic significance. We have addressed this question by using data from the SEER registry, which is a population based registry following approximately 10% population in the United States by collecting registry data from all health care facilities in large regions of the country. Patients included in the analysis had Stage 1 or 2 invasive breast cancer, at least 6 nodes examined, no positive nodes, and had a known histologic grade. The results of a multivariate analysis showed that for patients with tumors 1 to 50 mm size, a histologic grade of 1 (12% of all patients) conferred a 3.7 fold ($p < 0.00001$) better outcome that tumors with higher histologic grade. This result was not altered in patients with Stage 1 tumors (node negative with tumors 1 to 20 mm in size) in which patients with histologic grade 1 tumors (15% of the patients) had a 3.7 fold ($p < 0.00001$) better outcome.

To illustrate the absolute level of risk of breast cancer specific mortality estimates were made of mortality at 8 years of follow-up for 17,253 node negative patients with T1 or T2 tumor sizes. The values shown in this table are the breast cancer specific mortality at 8 years with 2 standard errors shown in parentheses.

	Grade 1	Grade 2	Grade 3&4
NN T1	1.7 (2.7)	4.0 (1.6)	10.0 (2.0)
NN T2	5.4 (12.6)	13.4 (3.7)	21.5 (3.3)
n=			
NN T1	2,382	5,784	4,724
NN T2	242	1,359	2,762

These results are particularly striking given the wide range of experience of the different pathologists that were performing the grading, and the lack of a uniform grading system. This suggests that features morphologic character of the breast cancer captured in grading systems, are among the most powerful prognostic indicators available. We conclude that histologic grade as reported by the average pathologist in the United States on pathology reports, is a very useful prognostic indicator.

132 Breast Cancer in Young Women Is Associated with a Worse Prognosis: A Case-Control Study.

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Several reports have indicated that young women (less than 35 or 40 years of age) have a worse prognosis than older women. We performed a case-control study in order to confirm this observation, and to determine whether this was attributable to increased microvessel density (MVD) or p53 expression in young women.

Methods: A retrospective search was performed of the Montefiore Medical Center Tumor Registry for women with stage I-III breast cancer who were 40 years or younger (cases) and had adequately preserved paraffin embedded tumor tissue. For each case, two or three control subjects older than 40 years were selected and matched for tumor size and nodal status. Immunohistochemistry was performed for evaluating microvessel density (CD34; Dako, Inc.) and p53 (Dako, Inc.) using previously reported methods. A Cox proportional hazard model was performed to examine the influence of age, MVD, p53 overexpression, and recognized prognostic factors on disease-free and overall survival.

Results: There were 26 cases (median age 36) and 72 controls (median age 64); the groups were well matched for median tumor size (30 vs. 25 mm), negative axillary nodes (54% vs. 61%), median tumor grade (6-7 for both), estrogen receptor positivity (62% vs. 78%). More cases received adjuvant chemotherapy (54% vs. 24%). There was no significant difference in median (39 vs. 40) or mean (41.4 vs. 46.2) MV count in cases compared with controls, and there was no difference in frequency of p53 overexpression (54% vs. 47%), defined as at least 10% of cells staining positively. The 5-year relapse free survival rate was significantly inferior for the cases compared with controls (45% vs. 80%; $p =0.0026$ log rank), which resulted in a significantly worse 5-year overall survival (45% vs. 74%; $p=0.0275$ long rank). In multivariate analysis, features associated with an increased risk of relapse included young age (hazard ratio [HR] 2.49; 95% confidence intervals [C.I.] 1.18-5.25; $p=0.02$) and positive lymph nodes (HR 2.44; 95% C.I. 1.12-5.30; $p=0.02$), and features associated with an increased risk of death included young age (HR 2.2; 95% C.I. 1.13-4.30; $p=0.02$).

Conclusions: We have confirmed previous reports demonstrating a worse prognosis for women 40 or younger with invasive breast cancer that is not explained by differences in recognized prognostic variables such as tumor size, grade, or axillary lymph node metastases. In addition, we found no correlation between young age and increased MVD or p53 overexpression.

133 Effect of Elevated Serum Carboxyterminal Telopeptide (ICTP) on Survival in Breast Cancer Patients with Only Bone Metastases.

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Considerable heterogeneity occurs in the survival of breast cancer patients with only bone metastases. Bone is made of Type I collagen fibrils that are crosslinked by pyridinium cross-links. Bone resorption releases the cross-links as free and peptide-bound (telopeptide) fragments. One of the markers of bone resorption, carboxyterminal telopeptide (ICTP), was evaluated as a prognostic marker. For this study ICTP levels were quantified in serum using an ICTP RIA from Diasorin, Inc. (Stillwater, Minn). This was a retrospective study using serum obtained from a subgroup of 111 patients with bone-only or with both bone and soft tissue metastases. These patients had participated in a double blind randomized study of second line hormonal therapy with a second-generation aromatase inhibitor (Fadrozole) vs. Megace. The mean followup for survivors was 832 days (range 597 - 1426). The normal serum ICTP level from a published report of 202 healthy postmenopausal women was 3.0 ± 1.6 ug/L (mean ± SD). The serum ICTP cutoff of 6.2 ug/L was established using the mean + 2 SD. Using this cutoff, 23 / 111 patients (21%) had elevated serum ICTP levels. Mean serum ICTP was 10 (6.3 - 21.2) ug/L in patients with elevated ICTP. Overall survival (OS) was significantly worse in patients who had elevated serum ICTP levels ($p = 0.004$). Kaplan-Meier estimate of median survival was 1.7 years in the elevated serum ICTP group as compared to 3.1 years in the not elevated serum ICTP group. Multivariate modeling for OS was performed including serum ICTP (≤6.2 vs >6.2 ug/L), disease-free interval (0 vs ≥2 yrs. and <2 vs ≥ 2 yrs.), and hormone receptor status. Serum ICTP level was a significant predictive factor for prognosis in the multivariate model ($p = 0.0012$).

In summary, elevated serum ICTP level is an important prognostic factor for decreased survival in breast cancer patients with only bone metastases.

134 The Impact of Young Age on Short-Term and Long-Term Clinical Course of Local Relapse in Early Breast Cancer.

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Following treatment for early breast cancer, a proportion of the patients will not experience a local relapse (LR) whereas the other patients are susceptible to undergo a LR that will mostly occur within 10 years of the initial treatment. Prognostic factors may be related to the time to failure for the susceptible patients (short-term component) and/or to the proportion of non susceptible patients (long-term component). Most survival analyses use the logrank test, which is however not well suited for characterizing the two components discussed above. An appropriate approach for tackling this problem consists of using survival models that incorporate a long-term survivor's fraction as a specific constituent.

The issue addressed in this work was to assess the short and long-term components when investigating the influence of age at diagnosis on LR. For this purpose, we present an original semi-parametric model that incorporates a long-term survivor's fraction. It allowed us to obtain test statistics for assessing the impact of a prognostic factor on the proportion of patients who are not susceptible for experiencing a LR and/or the distribution of time-to-relapse among those patients who are susceptible. The influence of young age on risk of LR was analyzed in 430 premenopausal patients with unilateral non-metastatic breast carcinoma with pathologic tumor size smaller than 20 mm and no lymph node involvement. All women were treated at the Curie Institute by combining limited surgery followed by irradiation. The median follow-up was of 103 months. The following age groupings were considered: 40 years old or less (104), between 41-49 (229) and 50 years old and higher (97). The results indicated that the probability of being non susceptible for experiencing a LR (long-term component) was significantly lower for young patients as compared to older patients. Moreover, among patients who were susceptible of experiencing a LR, the delay between initial treatment and LR was shorter in younger patients (short term effect). We think that new insights on prognostic factors effect for LR emerging from the proposed procedure may be helpful for individualized treatment approach.

135 Overexpression of Bcl-2 and Bax in Breast Cancer: Correlation to Established Prognostic Factors.

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Objectives: Overexpression of the antiapoptotic Bcl-2 is associated with a poorer prognosis in a variety of neoplasias. On the contrary, Bax, a proapoptotic member of the Bcl-2-family, seems to reflect a more favorable outcome. Bax has been shown to form heterodimers with bcl-2, the ratio of bcl-2 / bax determines the survival or death of cells. Taken together the ratio of Bcl-2 and Bax-expression is emerging as an important prognostic factor.

To correlate established risk factors such as nodal status, tumor size, receptor status, grading, Ki-67 and p53 with the expression of Bcl-2 and Bax we examined breast cancer specimens at primary diagnosis for overexpression of Bcl-2-family members.

Material and methods: 177 breast cancer samples, operated during 1997 and 1998, were analyzed for Bcl-2 and Bax by immunohisto-chemistry, using the ABC immunoperoxidase method for Bcl-2 as well as for Bax.

Results: 144 breast cancers (81,4%) were positive for Bcl-2, 73 (41,2 %) samples for Bax, 64 (38,8%) breast cancers were positive for both. Bcl-2 positive cancers were correlated to a negative nodal status ($p=0,061$), a positive receptor status, ($p< 0,05$), low grading ($p= 0,064$), low expression of p53 ($p<0,05$) and a low proliferation fraction Ki-67 ($p<0,05$). Bax positivity was not correlated with receptor status, nodal status or grading, but showed relation to high p53 ($p =0,073$) and low Ki-67.

Conclusion: The overexpression of Bcl-2 and / or Bax does not seem to correlate congruently to established risk factors and gives conflicting results. To determine the clinical value - eg. especially the prognostic impact - of our observations a long term follow-up of the described patients is necessary, which is currently in progress in our clinic.

136 Factors Influencing the Effect of Age on Prognosis in Breast Cancer: A Population Based Study.

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A recent paper by Kroman which showed improved survival for young women receiving chemotherapy has important implications for service delivery with increased amounts of chemotherapy required. The authors imply, but do not state explicitly, that only women at high risk received adjuvant chemotherapy. 36.3% of their 867 patients under 35 fell into the low risk group and it was among them that the excess mortality associated with not receiving chemotherapy was seen.

We used the Yorkshire Cancer Registry to see what the uptake of chemotherapy was for this group of patients over the 15 year period 1980 - 1994 and examined if this affected survival.

We found data on 1534 patients under age 35 of whom only 304 [19.8%] received adjuvant chemotherapy. The 5-year overall survival for this group was 60% [95% confidence intervals 54.8-65.8] for those receiving chemotherapy and 63% [60.6-66.0] for those who did not. Forty one patients presented with overt metastatic disease, if they are excluded from the analysis the 5-year survival rates increased to 63% and 64% respectively. The paper from Denmark does not give 5-year survival rates and thus direct comparisons are not possible.

There was no significant improvement in survival for those receiving chemotherapy for either the individual time cohorts or the group as a whole. A Wilcoxon [Breslow] test for equality of survivor functions showed no significant differences between the groups receiving chemotherapy and those who did not [$p=0.31$].

The percentage of chemotherapy usage in this age group increased from 8% in the years 1980-84 to 17% in 1985-1989 and to 32% in 1999-1994.

Reasons for the lower levels of chemotherapy in Yorkshire over this time can be related to the relative lack of surgical specialisation and lack of non-surgical oncology. The Danish patients were all included in trials where chemotherapy was used. We have previously shown large variations in chemotherapy and radiotherapy usage in Yorkshire with a deleterious effect for the whole breast cancer population receiving suboptimal therapy. Chemotherapy may only have been given selectively to the under 35s with conventional poor prognostic features and thus no overall effect seen.

With the end of high dose chemotherapy as an evidence-based option the optimum type of chemotherapy for this group of patients needs defining.

137 High Levels of Cathepsin D and c-erbB-2 Oncoprotein in Low Histological Grade Tumors Defines a Subgroup of Node-Negative Breast Cancer Patients with High Risk of Relapse.

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In node-negative breast cancer, the prognostic factors defined by the clinical and histological aspects of the tumor are commonly used but some patients with initially good prognostic relapse from their cancer and are poorly identified.

In the present study, based on prospective data of 197 tumors, we mesured cathepsin D (cath D, n=197), pS2 protein (n=125), c-erbB-2 oncoprotein (n=100) and epidermal growth factor receptor (EGF-R n=99) to better define the risk of relapse of node-negative patients in comparison with that defined by clinical and histological factors. Median follow-up in surviving patients was 75 months.

Univariate analysis indicated that patients with histological grade (SBR)III tumors had a much poorer prognosis than those with SBR I or II(p=0.0027 for relapse-free survival, p=0.0156 for overall survival (OS)). When the population of node-negative patients was divided by tertiles, high cath D levels showed a significant association with an early relapse (p=0.0316). Using cut-off values, patients with high cath D (≥25 pmol/mg protein) or c-erbB-2 oncoprotein(≥4HNU/µg protein)levels, had a significant worse relapse-free srvival (p=0.0147 and 0.0417 respectively). No prognostic information was supported by pS2 protein or EGF-R measurements.

In multivariate analysis, SBR grade, cath D and c-erbB-2 oncoprotein remained independent predictors of recurrence(p=0.005, 0.0361 and 0.0321).

By combining low levels of cath D and c-erbB-2 oncoprotein in SBR I or II tumors, we identified a subgroup of patients with a relapse-free survival probability of 100% at 6 years of follow-up. Morover,the subgroup of patients with SBR I or II and high values of both cath D and c-erbB-2 showed a prognosis as poor as that defined by SBR grade III alone, about 68% relapse-free survival at 6 years of follow-up.

In conclusion, the combination of conventional prognostic factor (hitological grading) and biochemical factors (cath D and c-erbB-2 oncoprotein) enables us to identify a subgroup of patients having an increased risk of relapse in a group (node-negative patients with low histological grade tumors) considered as good prognosis.

138 Morphological Characteristics of Metastatic Nodes Are Important as a New Prognostic Indicator.

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In patients with breast cancer, the number and level of node metastasis is known to be a good prognostic indicator and allows selection of those who might benefit from adjuvant medical treatment. But the relationship between morphological findings of involved node and prognosis has not yet been investigated. 1684 patients of invasive ductal carcinomas with undergoing mastectomy and lymph node dissection were reviewed. 710 cases were node positive. Morphology of positive nodes was microscopically checked and classified into 4 groups: C (only intra-capsular involvement), F (fat infiltration), L (lymphatic permeation), and V (vessel invasion). C means that cancer nest is limited within nodal capsule. F means that cancer invade to fat tissue beyond nodal capsule. L or V means cancer cells are detected in lymphatic or vessels adjacent to node. For example, a patient with C and L is classified as category CL. Over all survival and 10-year survival rates of each category and node negative (n0) were analyzed using Kaplan-Meier method and statistically significant difference was checked by log lank test. 15 categories were observed following the combination of 4 groups. Main four categories of 15 were C (360 cases), CF (130 cases), CFL (115 cases), and CFLV (25 cases). These 4 categories accounted for 88.5% (630/710).

OS in 4 categories and n0 were87.8% of n0, 74.7% of C, 60.2% of CF, 33.0% of CFL, and 23.9% of CFLV, respectively. Statistical differences between n0 and C, C and CF, CF and CFL, were significant ($p<0.005$). This result has made clear morphological characteristics of metastatic nodes to be important as a new prognostic indicator.

139 Locally Advanced Breast Cancer (LABC): Prognostic Variables Affecting Results.

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Three-hundred and sixty-seven LABC pts treated with a multimodality therapy entered two consecutive randomized trials performed within the framework of the North-West Oncology Group (GONO). In the first study 117 pts received either 3 courses of primary FAC (5-FU 600 mg/sqm, ADM 50 mg/sqm, 5-FU 600 mg/sqm day 1 every 21) followed by local-regional treatment (surgery and/or radiotherapy) and 6 courses of adjuvant chemotherapy (CT) consisting of 3 FAC alternated with 3 CMF (CTX 600 mg/sqm, MTX 40 mg/sqm, 5-FU 600 mg/sqm day 1 every 21) or the same program in which chemotherapy was preceeded by oral Dethylstilbestrol (DES 1 mg/day for 3 consecutive days). In the second study 150 pts underwent either a standard primary FEC (5-FU 600 mg/sqm, EpiDX 60 mg/sqm, CTX 600 mg/sqm day 1 every 21 days) or an accelerated FEC every 2 weeks with CSF. Clinical and pathological response rates to primary chemotherapy were superimposable in the two studies. On univariate analysis pts with clinical features of inflammatory breast cancer (IBC) had a significantly lower probability of response than pts who did not (p= .04); no other differences in response rates were observed between pre- and post-menopausal, estrogen (ER) and/or progesteron (PgR) receptor status, stage (IIIA vs IIIB) at diagnosis. The median PFS and OS of the whole group were 3,5 and 5,1 years respectively. At surgery, no relationships were observed between age, menopausal status, stage (IIIA vs IIIB), response to primary chemotherapy (pathological complete response, residual disease >/≤ 1 cm) and DFS or OS. Pts with. ER and/or PgR positivity had a better OS rates compared with pts with receptor negative tumors (p=.02 and p=.03 respectively); hormonal receptor positivity did not affect DFS. IBC correlates with OS (p=.0005) but not with DFS (.07). The number of positive nodes at surgery significantly predicts both DFS and OS (p=.0003 and p=.003 respectively). In LABC pts treated with a multimodality therapy hormonal receptor positivity at surgery significantly correlates with a better OS; IBC correlates with a poor outcome however is nodal status at surgery the strongest prognostic factor associated with PFS and OS. A multivariate analysis will be presented.

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140 Nuclear Grade and Tumor Marker Expression in Tubular Breast Carcinoma.

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Background: Tubular carcinoma (TC) is a special form of breast cancer characterized by a tubular growth pattern and is uncommonly seen in combination with cribriform (ICC) or not-otherwise-specified (NOS) breast carcinoma. In this study we examine the role of nuclear grade (NG) and effect of percentage of tubular component and tumor marker expression on the behavior of these tumors. Design: All grade I breast carcinomas and all cases stated to have tubular growth pattern over a 5-year period were reviewed. Nuclear grade was defined using the Scarff-Bloom-Richardson grading scheme. In borderline cases, nuclei in adjacent normal lobules were used to aid grading. Clinical data and follow-up information were obtained for correlation with nuclear grade and tumor marker expression. Results: Of the 95 cases of TC, 63 were “pure” TC, and 32 mixed TC. In the mixed TC, the non-tubular component was ICC in 21 cases, NOS type in 10, and both ICC and NOS types of breast carcinoma in one case. Although majority of the pure TC were nuclear grade 1 (88%), 50% of the mixed TC showed higher nuclear grade (NG 2). None of the cases were NG 3. Although NG did not correlate with tumor size, in both the pure TC and mixed TC, higher grade was significantly associated with lymphatic permeation (p=0.0001) and nodal metastases (p=0.0034). In mixed TC, higher nuclear grade was associated with a smaller tubular component. Also the presence of ICC component was associated with higher NG and aggressiveness characterized by recurrence or metastases. Of the 10 patients with recurrent or metastatic disease, 8 had NG2 (6 mixed; 2 pure TC). C-erbB2 and p53 expression was more frequent in NG2 for both pure TC and MTC ($p<0.05$). Four of 5 cases positive for ER/PR/p53/Cerb-B2, were MTC with NG2; 3 had recurrent or metastatic disease within 2 yrs. Conclusions: This preliminary study shows that high nuclear grade in TC at the time of presentation is associated with increased risk of nodal metastases and early recurrence. Higher NG was more common in mixed TC with smaller tubular component. Long-term follow-up studies are necessary to further elucidate the significance of these findings.

141 Biological Factors in Primary Breast Cancer (BC) of Young Women (≤ 35 Years).

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Young women with BC (≤ 35 years) have a worse prognosis than older patients. In multivariate analysis young age remains a significant predictor of recurrence and death after adjustment for other prognostic factors. To elucidate differences in biological factors between two age groups, we investigated primary BC of 27 patients ≤ 35 years and 27 patients between 50 and 70 years. All patients were matched according TNM classification. Proliferation markers MIB-1 and Topo-IIα, apoptosis markers p53, bcl2 and TUNEL, angiogenesis marker CD34 and metastasis marker CD44 variant 6 were investigated immunohistochemically (IHC). IHC assessment was performed in a blind fashion (age and clinical data). Of all specimens 500 cells were scored by 3 individuals. Mann-Whitney-U test and Komogorov-Smirnov tests were used.

Results: A significant correlation was found between MIB-1 and Topo-IIα ($p<0.05$). No correlation was found between other biological parameters. Proliferation markers MIB-1 and Topo-IIα were significantly higher in the younger group in comparison to elderly patients. P-values were respectively 0.015 and 0.01.

Conclusion: The dismal prognosis of young breast cancer patients seems to be correlated with a higher proliferation of tumor cells. This difference in proliferation rate should have implementation on therapeutic strategies in young breast cancer patients.

142 Amplification of HER-2 neu Predicts for Poorer Survival in Locally Advanced Breast Cancer.

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A number of studies have reported that amplification and/or overexpression of the HER-2 oncogene is associated with a poorer outcome in early breast cancer. Putative relationships between response to therapy and outcome have been suggested as in part responsible for this association, but individual tumour response cannot be ascertained in most of the reported series. We have recently reported the outcome for a series of over 70 patients with locally advanced breast cancer, treated at a single institution with infusional 5-FU based neo-adjuvant chemotherapy for 12 weeks {The Breast, Davidson et al 1999}. HER-2 neu gene amplification was assessed in paraffin embedded formalin-fixed sections using the Pathvysion FISH diagnostic technique.

To date 32 patients (mean age 47 years) have been assayed, with 14 (44%) demonstrating HER-2 neu gene amplification. Median follow-up is 6 years, and 28/32 (88%) received an anthracycline. There were no significant differences in patient age, nor the proportions of tumours that were inflammatory, ER+ve, or node positive. Similarly there were no differences in tumour response according to HER-2 neu status. There were no significant differences in outcome by tumour size, clinical response to therapy, node status at surgery, or use of adjuvant tamoxifen. The survival of patients whose tumours had amplificaiton of HER-2 neu was significantly worse ($p < 0.02$).

This study reports a high incidence of HER-2 neu positivity in locally advanced breast cancers, irrespective of the presence of inflammatory cancer, which although not influencing tumour response, was still associated with a poor prognosis. This has important implications for the management of these patients.

143 Traditional and New Prognostic Factors in Locally Advanced Breast Cancer.

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Introduction: Patients with 10 or more tumor infiltrated axillary lymph nodes or extracapsular node infiltration have an unfavourable prognosis. We report on evaluation of traditional and newer prognostic factors of patients randomized in a prospective trial on dose-intense chemotherapy.

Methods: Between 1993 and 1998, 182 patients were recruited and randomly assigned to dose-intense chemotherapy (diEC 120/600 *4, q14d) in comparison to a conventional regimen (EC 90/600 *4, q21d CMF*3). Within this patient cohort, traditional and new prognostic factors, uPA, PAI-1 (ELISA, American Diagnostica), and c-erbB-2 overexpression (IHC; Ab 3B5, Oncogene Diagnostics) were determined. In 175 evaluable patients, 59 recurrences and 39 deaths were observed. Median follow-up of surviving patients is 42.1 months (2.8 to 78.0 months).

Results: In univariate analysis of disease-free survival (DFS), factors for risk of recurrence were number (>10) of tumor infiltrated axillary lymph nodes (LNN; p=0,0001), age <40 years(p=0.0474), negative progesterone receptor status (PgR; p=0,006), vessel invasion (VI; p=0.0031), grade 3 (G3; p=0,0085), and extracapsular lymph node infiltration (p=0.009). In multivariate analysis of DFS, LNN (RR=2.88; p=0,0002), PgR (RR=2,16; p=0,0036), and age (RR=2.20; p=0,0158) were of independent prognostic value. New tumorbiological factors, uPA, PAI-1, and c-erbB-2 had no prognostic value in these analyses. Also in regression tree analyses (CART), number of infiltrated lymph nodes, PgR and age were the most important prognostic factors; in addition G3 and VI indicates unfavourable prognosis in distinct subgroups.

Conclusion: In locally advanced breast cancer patients, number of tumor infiltrated axillary lymph nodes, age and progesterone receptor status determine the prognosis. Further biological factors are of minor value.

144 18F-FDG-Positron Emission Tomography and Breast Cancer: Does FDG-Uptake Correlate with Prognostic Markers?

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The aim of this study was to evaluate a possible correlation of preoperative FDG-PET in human breast cancer and the prognostic markers Ki-67, c-erb B2, p53, estrogen-/progesteron receptor status and tumor grading. **Methods:** 42 female patients with breast cancer were included into this prospective study. A high resolution full-ring scanner (Siemens ECAT HR+) was used for preoperative PET imaging. The FDG-uptake of mammary tumors was calculated as tumor to background ratio (TBR). In resected cancer tissue specimens, proliferation fraction was evaluated by Ki-67 immunostaining. Additionally, immunostaining of the prognostic markers c-erb B2, p53, progesteron- and estrogen-receptors was performed. Hematoxylin-eosin-stained sections were used for tumor grading. For evaluation of possible correlations between FDG-uptake and prognostic markers, linear regression analysis was performed. **Results:** In ductal breast cancer, FDG-TBR was 14.6 (median 5.9, range 1.6-122.7), in lobular breast cancer 2.7 (median 4.8, range 1.4-22.7). Mean proliferation fraction (% Ki-67 positive cells) was 12% (median 10%, range 0-70%). 13 carcinomas did not show positive Ki-67 staining. Statistical analysis indicated a positive correlation of FDG-uptake and proliferative index (p=0.0001). On the contrary, there was no correlation of FDG-uptake and c-erb B2 (p=0.34), p53 (p=0.97), tumor grading (p=0.09), estrogen- (p=0.08) and progesteron-receptor status (p=0.31). **Conclusions:** In ductal breast cancer, FDG-uptake is significantly higher as compared to lobular cancer ($P<0.05$). The positive correlation of FDG-uptake and proliferative fraction indicates that FDG-uptake might correlate with prognosis. On the other hand, the lack of correlations to the other examined prognostic markers does not support this hypothesis.

145 Micrometastases in Axillary Lymph Nodes Detected by RT-PCR as a Valuable Prognostic Factor in Node-Negative Breast Cancer Patients.

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Introduction: We evaluated the clinical significance of micrometastases in axillary lymph nodes (AxLNs) of breast cancer patients for prediction of the prognosis. Using RT-PCR technology, we examined the micrometastases in AxLNs of patients whose long-term clinical outcomes were known.

Material and Methods: Archived formalin-fixed paraffin-embedded AxLN specimens from 129 node-negative breast cancer patients diagnosed by routine hematoxylin and eosin staining between 1986 and 1990 were subjected to carcinoembryonic antigen (CEA)-RT-PCR analysis. RNA extraction from formalin-fixed specimens and reverse transcription was efficiently carried out according to our original methods (Nucleic Acids Research, 27: 4436-43, 1999). After median follow-up period of 105.6 months, disease-free and overall survival were estimated by Kaplan-Meier method, and then we analyzed associations with various prognostic factors as well as the effect of micrometastases in AxLNs on patient prognosis.

Results: Micrometastases were detected in 40 of 129 (31.0%) node-negative breast patients. Ten-year disease-free and overall survival was respectively 87.6% and 93.7% in patients without micrometastases, whereas patients with micrometastases had significantly worse clinical outcome (disease-free; 66.1%, P=0.0008 by log-rank test, overall; 67.8%, p=0.0024). Adding this micrometastases in AxLNs, estrogen receptor status, histologic type, nucleic grade, lymphatic invasion and vascular invasion were assessed as important prognostic factors in univariate analyses. By multivariate analyses, AxLN micrometastasis was revealed to be an independent and significant predictor of outcome. The hazard ratio was 3.992 (95% CI 1.293-12.323, p=0.0161) for relapse and 4.293 (95% CI 1.043-17.675, p=0.0436) for cancer-related death.

Conclusion: The molecular staging of AxLNs using RT-PCR technology is useful for the prediction of clinical outcome in early breast cancer patients and can provide a powerful and sensitive complement to routine histopathologic analysis.

146 Efficacy of Prophylactic Mastectomy in Unaffected BRCA1/2 Mutation Carriers: First Prospective Study.

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Women with a BRCA1 or 2 mutation have a high lifetime risk (55-85%) of breast cancer (BC). They can opt for regular surveillance, prophylactic mastectomy, oophorectomy and chemoprevention. Recently we reported that 51% of our healthy BRCA1/2 mutation carriers without BC opted for bilateral total prophylactic mastectomy (PM) (Lancet, June 10, 2000). In the present prospective non-randomized case-control study we investigated the efficacy of PM in comparison with that of regular surveillance. The mean age of 69 healthy BRCA1/2 mutation carriers opting for PM (36.8, range 23-56 yrs) did not significantly (p=0.19) differ from all our 70 female carriers (38.9, range 19-64 yrs) opting for regular surveillance. The median follow-up was similar for both groups (2.03 and 2.02 yrs). The spectrum of different BRCA1 (n=120) and BRCA2 (n=19) mutations was comparable in both groups. In the PM group 42 women underwent prophylactic oophorectomy vs 32 in the surveillance group. During the study chemoprevention was not available.

Results: After PM we did not observe any case of breast cancer, while in the surveillance group 8 incident cases with BC (12%) in addition to one prevalent case were detected during follow-up. The 5 year actuarial breast cancer rates were 0 and 29% in the PM and surveillance group respectively (p=0.02). This difference remained significant (p=0.04) when adjusted for age and oophorectomy. The age of the 8 incident cases varied between 23-52 yrs, tumor sizes between 7-40 mm (4/8 being node-positive) while all tumors were ER-negative. One patient (28 yrs) died 26 months after diagnosis. In the surveillance group the incidence rate was 4.4% per year and 2.5 times (95% CI: 0.6-15.6) higher than expected for BRCA1/2 mutation carriers (8 vs 3.2 cases).

In conclusion: Prophylactic mastectomy reduces the risk of breast cancer significantly already on the short-term, while in the surveillance group an unexpectedly high number of incident cases was detected. (Supported by grant DDHK 95-953 of the Dutch Cancer Society).

147 Study of the Biological Effects of the Aromatase Inhibitor Letrozole in Healthy Postmenopausal Women: Rationale for Prevention.

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Aromatase inhibitors are becoming accepted as first line treatment for metastatic breast cancer, are in clinical trials versus tamoxifen for the adjuvant treatment of early breast cancer and are being considered for evaluation in the prevention setting. Third generation inhibitors such as letrozole are well tolerated and anticipated to have fewer thromboembolic complications and no endometrial proliferative effects in contrast to tamoxifen. Recent data suggesting that oestrogens may have direct mutagenic effects provide an additional rationale for using aromatase inhibitors in prevention. There is some concern that the profound oestrogen deprivation may, however, have detrimental metabolic effects on tissues such as bone. The primary aim of the present study was to evaluate the effects of letrozole on normal epithelial cell proliferation (considered an intermediate endpoint of a likely prophylactic effect) in postmenopausal breast tissue. It was designed to recruit 30 postmenopausal, healthy volunteers not on HRT who had been treated at The Royal Marsden Hospital for benign breast disease, DCIS or LCIS. They were to take 2.5mg/day of letrozole for 12 weeks with blood sampling and ultrasound guided core-biopsy of their normal breast before commencement and at the end of the 12 weeks. 335 women were initially contacted by letter of which 208 (62%) have replied to date. 140 were recontacted by telephone and about 50% proved to be ineligible. 41 of the 61 who requested full information packs replied, 18 (30%) agreed to participate and 19 (31%) declined. 41/208 with no telephone contact were sent information packs directly; 4 (10%) accepted and 6 (15%) refused. 19 women have commenced the study to date and only one participant has withdrawn. From the initial biopsies on these 19 women only one had to have repeat sampling because it was estimated that there would be to few cells for analysis of tissue biomarkers. Biological data collection will be completed during the next 5 months. CONCLUSION. This novel study design will afford biological data on aromatase inhibtors as potential chemopreventives. The ultrasound-guided core-biopsy can give good tissue yield from normal postmenopausal breast and has been well tolerated proving this type of study is feasible.

148 Chemopreventive and Chemotherapeutic Effects of Tamoxifen and 9 Cis Retinoic Acid in the MNU-Induced Rat Mammary Tumor Model.

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A combination of low doses of the antiestrogen/estrogen tamoxifen (0.13 or 0.4 ppm in diet) combined with the RAR/RXR pan agonist 9 Cis retinoic acid (9 CRA) (60 ppm) were tested as preventive and therapeutic agents in the MNU-induced, hormonally responsive, rat mammary tumor model. Treatment beginning shortly after tumor initiation (55 days of age) and continuing until the end of the experiment (175 days of age) profoundly decreased tumor incidence (>70%) and tumor multiplicity (>90%). In contrast limited treatment from 55-115 days of age followed by control diet resulted in limited effects on tumor multiplicity and none on tumor incidence. When control MNU treated rats with small palpable mammary lesions (5-8 mm diameter) were treated with this regimen minimal effects on tumor growth were observed. In contrast this regimen did cause significant decreases in proliferation and increased apoptosis in the lesions. These results demonstrate that the doses required for therapeutic efficacy and for preventive efficacy for this drug combination are distinctly different and that limited treatment (Days 55-115) with this drug combination had minimal effects on tumor development. (Supported In Part by NCI Contract NO1-CN-85076)

149 Phase II Chemoprevention Trial of DFMO Using the Random FNA Model.

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An NCI-funded, randomized, double-blind Phase II chemoprevention trial of 6-months of α-DiFluoroMethylOrnithine (DFMO, 500 mg/m2/day) vs. placebo was conducted using random periareolar fine needle aspiration (FNA) to provide ductal epithelial cells to assess for surrogate endpoint biomarkers (SEBs) of drug efficacy. We recruited high risk women who had random FNA cytologic evidence of epithelial hyperplasia +/- atypia. Cytology changes and change in EGFR, PCNA, and p53 expression semiquantitated via immunocytochemistry index scores were examined, as well as changes in breast density, serum IGF-1/IGFBP3 ratio, and urinary polyamines. We enrolled 119 women in 23 months. Treatment was well-tolerated with a 41% incidence of grade 2 possible study agent related side effects but only one possible grade 3 adverse event. The most frequent complaint was auditory (usually tinnitus) in 33/119 (28%) of subjects. All but 5 women completed the 6 month study, with pre- and post-study FNA specimens. Favorable modulation of cytologic morphology was seen in 33/114 women. A decrease in EGFR staining and mammographic density was also observed. No consistent changes were exhibited for breast tissue PCNA or p53; or for serum IGF-1/IGFBP3 ratio. Once the study is unblinded in September, urinary polyamine levels will be assessed as drug effect markers and a comparison of placebo vs. DFMO for all study biomarkers will be performed. In summary, a clinical Phase II chemoprevention trial in which high risk women undergo random FNA before and after study agent administration has been completed. The model has proven feasible with rapid recruitment, minimal drop-out, and collection of pre-post FNA specimens in >95% of subjects. It remains to be seen whether DFMO at a dose of 500 mg/m2 daily for 6 months significantly modulates breast FNA cytology, EGFR expression, breast density, and/or polyamine levels.

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150 Safety of a Gonadotropin-Releasing Hormone Agonist (GnRHA)-Based Hormonal Chemoprevention Regimen for Young Women at High Genetic Risk for Breast Cancer.

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Germline mutations in breast cancer genes such as *BRCA1* confer significantly increased risk for early onset breast cancer. Breast tissue density limits the usefulness of mammography as a surveillance tool in young women. There is overwhelming evidence for the role of ovarian hormones in the etiology of breast cancer and an association with breast density. Menopausal symptoms and concerns about impaired fertility and safety limit the utility of SERMs as chemopreventives in young women. The purpose of this study is to examine the effects of a one-year trial with an intranasally administered drug combination of the GnRHA, deslorelin, with partial replacement of 17β-estradiol (E2) and testosterone (T) in women at high genetic risk of breast cancer. Intermittent oral medroxyprogesterone acetate is given to protect the endometrium. The estrogen exposure remains lower than at any time in the normal menstrual cycle. The regimen is hypothesized to reduce mammographic densities as well as reduce breast cell proliferation. If these hypotheses are true, the regimen will not only result in enhanced mammographic screening (and facilitate detection of asymptomatic cancers), but is likely to significantly reduce the risk of breast cancer. It is predicted that such a regimen should reduce lifetime breast cancer risk by one third if used for 5 years and by more than 70% if used for 15 years. Preliminary results from this study of young women with *BRCA* gene mutations indicate that deslorelin decreases breast density, similar to the results of a prototype regimen that significantly (p=0.0073) decreased mammographic density compared to placebo (Spicer et al, 1994). Safety data indicate that bone density is stable on this regimen and that the return to normal menses is relatively prompt upon cessation of the drug (mean 31 days for 50 subjects treated with deslorelin in a fibroid treatment trial). Thus, deslorelin can be combined with E2 and T in a nasal spray at doses that are sufficient to maintain a woman in good health and still achieve decreased breast density, and the combination is a promising regimen for breast and ovarian cancer risk reduction.

151 Identifying Subjects for Breast Cancer Risk Reduction: An Epidemiologic Approach.

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Epidemiologic studies identify factors that increase the risk of developing invasive breast cancer (IBC), and quantitative models permit calculation of the probability of developing IBC. Interventions evaluated in prospective studies can reduce the chance of developing IBC, but each is associated with known morbidities. The important risk factors (in addition to age) for IBC based on their prevalence in the population are: age at menarche, age at first live birth (AFLB) or nulliparity, benign breast pathology (BBD, including atypical hyperplasia [AH] and lobular carcinoma in situ [LCIS]), family history of IBC in first-degree relatives, and/or the presence of predisposing genetic mutations (N Engl J Med 1992; 327:319-328). We have shown that population surveys and cohort studies allow estimation of both the prevalence of important risk factors and the number of women who are at risk (Prev Med 1991; 20:86-100).

Breast cancer risk factor	Prevalence in population	No. of women ages 35-59 with risk factor*
Early menarche	0.16	7700
Nullip/AFLB>30	0.21	10000
BBD	0.15	7200
Family history	0.08	3800
BRCA1/2	0.01	480
AH/LCIS	0.01	480
Total		29660

(*Population in thousands; data from US Census, May 2000) Prophylactic mastectomy and/or oophorectomy, and the use of SERMs reduce risk of IBC by 30-50% or more in selected population subgroups. We used the algorithms developed by Gail et al (J Natl Cancer Inst 1999; 91:1829-1846) and other published estimates of outcome event rates to calculate net benefit (or harm) for each intervention. Prophylactic surgery provides net benefit, although risk of heart disease, osteoporosis, and psychological morbidity are increased. Tamoxifen offers age-dependent net benefit to women younger than 50 and to moderate-risk women between the ages of 50 and 59 years. Net benefit also occurs following surgery in mutation carriers; the effect of SERMs in this population is uncertain. Targeted interventions can reduce incidence of IBC by 30-50% and should be considered systematically for one-half of the 48 million US women between the ages of 35-59 years who have at least one risk factor for IBC.

152 A Randomized Trial of Fenretinide in HRT Users Using IGF-I as a Surrogate Biomarker.

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Hormone Replacement Therapy (HRT) improves quality of life and may reduce mortality in postmenopausal women, but it increases the risk of breast cancer. Moreover, the effect of HRT on breast cancer risk may be different according to the route of administration. For example, transdermal HRT (tHRT), in contrast to oral HRT (oHRT), leaves unchanged plasma Insulin-like Growth Factor-I (IGF-I), a known mitogen for the breast gland. Fenretinide (4-HPR), a vitamin A derivative, has been shown to decrease IGF-I in premenopausal women with breast cancer and this effect is associated with a reduction of second breast cancer incidence.

We started a clinical trial in healthy postmenopausal women that are randomized to receive either transdermal or oral HRT with placebo or 4-HPR for one year. The primary endpoint is the change in plasma levels of IGF-I. Other biomarkers are: mammographic density, hormone and lipid profile, C-reactive protein, dysplasia and proliferation in cytology through ductal lavage/FNA. As of May 15, 2000, 213 women have been randomized. Main subject characteristics (mean ± SD) on tHRT and oHRT are respectively: age 52.3 ± 3.2 vs 53.1 ± 3.3; Gail 5-y breast cancer risk (%) 1.2 ± 0.3 vs 1.3 ± 0.4; BMI (Kg/m^2) 24.9 ± 4.0 vs 24.2 ± 3.2. Mean time of drug exposure is 239±131 days, while mean time of observation is 264±146 days. Overall compliance is >90% in nearly 90% of the subjects. Most frequent side effects were vaginal bleeding, diminished dark adaptation and mammary tension. Two subjects had a severe adverse event (1 VTE and 1 unattended bleeding). We noted a high compliance and a low drop-out rate, without major adverse events. Follow-up is continuing and unblinded results are awaited shortly.

153 **Effects of Dietary Flaxseed in Women with Cyclical Mastalgia.**
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Cyclical mastalgia is a common, often distressing disorder among women and has variably been associated with breast cancer risk. Although hormone treatments, such as tamoxifen, may be helpful, they often cause unpleasant side effects and the risks associated with long-term use of hormonal therapy in premenopausal women are unknown. Dietary flaxseed is an attractive alternative for controlling the symptoms since flaxseed is a rich source of n-3 fatty acids and mammalian precursors of lignans, which are structurally similar to tamoxifen and may act to antagonize the action of endogenous estrogens. Therefore, the objective of this study was to examine the clinical and biological effects of dietary flaxseed in women with cyclical mastalgia. 116 premenopausal women with severe cyclical mastalgia over a pre-study period of 6 months were randomized in a double-blind manner to either a 25 g flaxseed containing muffin (56 women) per day or a placebo muffin (60 women) and followed up for 4 (one pretreatment) menstrual cycles. Visual analogue scales (VAS) were used for assessment of breast pain, swelling and lumpiness at each menstrual cycle. Subjects also recorded the severity of their breast pain on a daily pain chart. At the end of each menstrual cycle, blood and urine samples were collected for fatty acid, hormonal and lignan analyses. Results showed that breast pain was alleviated in both treatment groups but the median reduction of VAS score was significantly greater ($p=0.0394$) in flaxseed group (31.75) than that in placebo group (17.00) at the end of 3 treatment cycles. No significant side effects were observed. Urine lignans and n-3 fatty acids were increased in the flaxseed group indicating good treatment compliance and their potential role in the observed effects. Correlation between hormones and breast pain was done. It is concluded that flaxseed is effective in relieving symptoms of cyclical mastalgia without significant side effects and might be considered as an alternative treatment for cyclical mastalgia. Its putative mechanism of action maybe via the anti-estrogenic effects of the lignans.

154 **The Women's Healthy Eating and Living (WHEL) Study: A Nutritional Intervention Study in Breast Cancer Survivors.**
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The WHEL study is an innovative nutritional intervention study initiated in 1995 to test the hypothesis that a diet rich in plant based foods is associated with a longer breast cancer event free interval in breast cancer survivors. Cross-cultural comparisons demonstrate a strong association between dietary patterns and breast cancer risk. The most compelling evidence indicates the importance of protective factors, largely unidentified, primarily found in vegetables. Longitudinal studies have identified initial levels of serum β carotene, a marker of vegetable intake, as a strong predictor of subsequent cancer events. To this end, the WHEL study has targeted a 75% increase in serum β carotene, to be obtained through food sources, in its study design.
Randomization will be completed by 11/00. 3000 women will follow either the NCI guidelines (5 servings of fruits and vegetables/day, 20 grams of fiber, and 30% calories from fat) or an intervention (WHEL) diet in which the goals are approximately twice those of the NCI diet. Women who are within 4 years of their breast cancer diagnosis, stages I (≥1cm)-IIIA, and have completed planned adjuvant chemotherapy or radiation are eligible and will follow the diets for 4 years. Blood and tissue for correlative studies, dietary recalls, physical measurements, and quality of life assessments are obtained throughout the study.
Results to date: At baseline, dietary index scores for both groups are identical. At 6 months there is a dramatic increase in the vegetable intake in the WHEL group, which is maintained over 36 months. This is the first study of its kind to achieve such a significant change in vegetable consumption. Validation of dietary self- reports is reflected in serum levels measured: a 200% increase in circulating α carotene, a 75% increase in β carotene, and a 30% increase in lutein. Power calculations project the ability to detect a 17% difference in breast cancer events over the next 4 years.

155 **Comparison of Dietary Assessment Methods in a Low Fat Dietary Intervention Program for Women at High Risk for Breast Cancer.**
Simon MS, Uhley V, Djuric Z, Lababidi S, Depper J, Kresge C, Klurfeld DM, Heilbrun L. Hematology/Oncology, Barbara Ann Karmanos Cancer Institute, Detroit, MI.
The Food Frequency Questionnaire (FFQ) is the most commonly utilized instrument for the assessment of dietary fat intake, but its validity as a method of choice for dietary assessment among individuals following a low fat diet is unclear. We evaluated the agreement of nutrient estimates derived from FFQ, 24-hour dietary recall, and 3-day food records that were obtained from 104 participants in a randomized trial of a low fat dietary intervention (15% caloric intake from fat vs. usual diet) for women at an elevated risk for breast cancer. Comparisons were made for total caloric intake, % calories from fat and total fat (g) after 1 year on study. For the most part, estimates derived from the FFQ were greater than those derived from recalls or food records with means estimated by FFQ ranging from 5 to 25% higher. Instrument agreement was reported as Pearson correlation coefficients and 95% CI's. Correlation was assessed using standard methods based on a null hypothesis of no agreement between dietary instruments, as well as by a newer methodology based on a hypothesis that the instrument results should be in agreement. (Hebert and Miller, 1991) Estimates derived from FFQ data were significantly correlated to food record estimates for women on a usual diet, and only significantly correlated for women on a low-fat diet for % calories from fat. Assuming a null hypothesis of instrument agreement, we only found significant correlation between recall and record data for total caloric intake ($r=0.672$) for women on the low-fat diet, and showed no significant correlation between FFQ and either recall or record data for any dietary variable. The results of this analysis do not support the use of FFQ data alone to evaluate dietary intake among women participating in a low-fat dietary intervention program.

156 **Weight Loss in Obese Breast Cancer Survivors: Novel Strategies.**
Djuric Z, DiLaura N, Jenkins I, Mood D, Jen C, Hryniuk W. Karmanos Cancer Institute, Detroit, MI.
Obesity is a major health problem in women who have had breast cancer. Treatment of obesity, however, has been largely unsuccessful over the long-term. We started a pilot study to develop an individualized approach towards weight loss in obese breast cancer survivors. This approach is being tested either alone or combined with the Weight Watchers® program. Forty eight obese breast cancer survivors (BMI 30-45) have been enrolled. At baseline, a psychiatric evaluation was obtained for each woman and questionnaires on diet, health, exercise and psychosocial factors were administered. Blood samples are being collected for analysis of biomarkers of cancer risk. Preliminary data are available at the 3-month time point for 34 women. Weight loss in the control group was only 1.9 + 8.9 lbs. (n=9) and body fat was increased 0.30 percentage points even though the women reported large decreases in caloric and fat intakes. Weight loss was: 9.1 + 8.4 lbs. (n=6) in the individualized group, 8.0 + 7.9 lbs. (n=9) in the Weight Watchers group, and 17.3 + 7.4 lbs. (n=8) in the combination diet group (the latter was significantly different than control or Weight Watchers alone). Body fat decreased in the intervention arms: 2.30 percentage points in the individualized, 0.3 in Weight Watchers and 1.5 in the combination arm. Data on 7 women in the combination arm at 6 months indicate a mean weight loss of 26 pounds representing 13% of initial body weight, which exceeded our goal of 10%. This data indicates that dietary change may be easiest to achieve when dietary counseling is done using both the group setting and individualized contact. This also increases the frequency of contacts, and was effective even though most of the contacts were by phone.
Supported in part by The Weight Watchers® Group.

157 Biological Effects of Dietary Flaxseed in Patients with Breast Cancer.

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Epidemiological studies and biological properties of mammalian lignans derived from plant precursors (phytoestrogens) suggest that they may have anticancer potential. Flaxseed, the richest source of mammalian lignan precursors, has previously been shown to reduce the mammary tumor number and growth of established tumors in rats. The aim of this study was to examine, in a randomized double blind, placebo controlled, prospective clinical trial, the effects of dietary flaxseed on tumor biology, urinary lignan excretion and side effects in patients with newly diagnosed breast tumors. Patients were randomized to either a 25g flaxseed containing muffin (6 pre-, 17 post-menopausal) or a control (placebo) muffin (4 pre-, 12 post-menopausal). At initial diagnostic core biopsy and at definitive surgery, (a) tissues were analyzed for rate of tumor cell proliferation (Ki67 labeling index and score), c-erB-2 expression, and estrogen (ER) and progesterone (PR) receptor levels, (b) 24-hr urine samples were collected and analyzed for lignans, and (c) 3-day diet records were analyzed for nutrient intake. Side effects were monitored. Mean treatment times were 39 and 38 days in the placebo and flaxseed groups, respectively. In postmenopausal women, significant reductions (21-33%) in Ki67 labeling index ($p<.036$) and scores ($p<.029$) and in the c-erB-2 expression ($p<.040$) were observed in the flaxseed group but not in the placebo group. These changes are comparable to those seen with tamoxifen using similar study protocol. No significant differences in the ER and PR levels and in caloric and macronutrient intakes were seen between groups and between pre- and post- treatment periods. Significantly higher post-treatment urinary lignan excretion was observed in the flaxseed group compared with placebo and with pre-treatment levels. No significant adverse effects of flaxseed were reported. This study showed, for the first time, the potential of dietary modification with flaxseed and its components such as the lignans, in reducing tumor growth in patients with breast cancer comparable to the effects seen with preoperative tamoxifen.

158 Evidence for TGFβ-Mediated Growth Inhibition by Genistein in Human Mammary Epithelial Cells.

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Genistein (5,7,4'-trihydroxyisoflavone: GEN), the principal isoflavone in soy, has a variety of effects in models of human chronic disease, including inhibition of growth factor-stimulated proliferation of many human tumor cell lines. However, its mechanism of action in chemoprevention has not been defined. Transforming growth factor β (TGFβ) also inhibits proliferation of many epithelial cell types. In this study we examined whether GEN modulates the expression or actions of TGFβ. Human mammary epithelial cells (HME) were cultured over a 3-day period in a growth medium supplemented with bovine pituitary extract (BPE), epidermal growth factor (EGF, 30 ng/ml) and varying concentrations of GEN (0-100 μM). Using the sulforhodamine B assay, it was shown that GEN significantly inhibited BPE/EGF-stimulated HME cell proliferation with an IC_{50} of 12 μM; in contrast, whereas TGFβ at 4 pM caused a 40% decrease in proliferation, further increases in inhibition were not observed at higher concentrations of TGFβ. A sandwich enzyme-linked immunosorbent assay for total TGFβ showed that conditioned medium from HME cells exposed to GEN (1-10 μM) contained dose-dependent increases (range 3.9-7.4 pM) in TGFβ concentration which reached a maximum after two days. GEN-induced TGFβ in the conditioned medium was active as demonstrated using mink lung cells transfected with a plasminogen activator inhibitor-promoter (which contains a TGFβ-response element)-luciferase reporter gene construct. Analysis of the data in the two assays revealed that 67% of the TGFβ in conditioned medium was biologically active. Although GEN inhibits proliferation of human breast cancer MCF-7 cells, it did not induce TGFβ secretion by these cells. In summary, the magnitude of TGFβ production in response to GEN suggests that the mechanism of action of GEN in chemoprevention of breast cancer may involve TGFβ-inhibition of mammary epithelial cell growth. However, different mechanisms must occur in breast cancer cell lines.

159 A Randomized, Placebo-Controlled, Double-Blinded Clinical Trial of a Soy Beverage in the Treatment of Hot Flushes in Breast Cancer Survivors.

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Menopausal symptoms are often induced or worsened by breast cancer treatments including chemotherapy and tamoxifen or provoked by the discontinuation of HRT after diagnosis. There are a lack of safe and effect treatments for menopausal symptoms in cancer survivors and HRT is seldom advised due to the possible risk of stimulating cancer growth. Thus, the purpose of this study was to evaluate the acceptability and effectiveness of a soy beverage (containing ~90 mg of plant estrogens) in the treatment of menopausal symptoms in postmenopausal women with a history of treated breast cancer. Women were eligible if they had breast cancer; were symptomatic for hot flushes, were >4 months since completion of treatment (may be taking tamoxifen), and postmenopausal. Each participant completed a validated daily diary for 4 weeks prior to study entry, and for 12 weeks after randomization while consuming 500ml/day of a soy or placebo beverage. At baseline, 4, 8, and 12 weeks, women completed a questionnaire to measure the frequency of soy, alcohol and caffeine consumption, and the use of alternative therapies. The measurement of serum FSH, alkaline phosphatase and γGT were obtained at baseline, and an additional sample of serum and urine was stored at baseline, 6 and 12 weeks for future analysis. A total of 163 women were recruited and 111 women have completed participation. The study will be unblinded in June and the results available for presentation. *Funded by The Canadian Breast Cancer Research Initiative.*

160 Recombinant Human Chorionic Gonadotropin (r-hCG) Reduces the Incidence and Progression of Mammary Tumors in the Rat.

Russo IH, Slater C, Lareef MH, Mihaila D, Ao X, Quillen DP, Arulanandam ARN, Russo J. Fox Chase Cancer Center, Philadelphia, PA; Serono, Randolph, MA.

The life-time risk of breast cancer is decreased by full-term pregnancy at a young age. In the rat, either pregnancy or administration of urinary (u-) hCG lowers the incidence and progression of 7,12-dimethylbenz(a)anthracene (DMBA)-induced mammary tumors. To investigate if the anti-tumor effects of u-hCG are due to hCG, a preparation of r-hCG (Serono) was evaluated for its prophylactic and therapeutic effects in DMBA-induced mammary tumors in rats. Intact virgin age-matched Sprague-Dawley rats received a single-dose of DMBA intragastrically (8mg/100g body weight) to induce mammary carcinogenesis at age 45 days (therapeutic treatment) or 87 days (prophylactic treatment). Animals were given daily intraperitoneal injections of placebo, r-hCG (5μg), or u-hCG (100 IU). Rats received prophylactic treatment with hCG between the ages of 45-66 days, resulting in a 84-89% reduction in DMBA-induced turmors/animal compared with placebo-treated animals. Rats received therapeutic treatment of hCG either early, between the ages of 66-106 days, when DMBA induced micro tumors, or late, between the ages of 106-146 days, when tumors are readily palpable, resulting in a 61-66% and 32-40% reduction in tumors/animal, respectively, compared with the placebo-treated group. Mammary tissue harvested at necropsy from hCG-treated animals (therapeutic treatment) showed a significant increase in apoptosis and inhibin induction as determined by immunohistochemistry analysis.

	Prophylaxis			Early treatment			Late treatment		
	n	Tumor incidence	Tumors /animal	n	Tumor incidence	Tumors /animal	n	Tumor incidence	Tumors /animal
Placebo	49	45%	0.89	49	98%	5.6	40	98%	5.7
r-hCG	49	8%***	0.10	49	78%*	2.2	37	89%**	3.9
u-hCG	50	12%***	0.14	46	65%***	1.9	36	89%**	3.4

*p<0.05; **p<0.01; ***p<0.001 vs. placebo.

In conclusion, these studies confirm that the prophylactic and therapeutic effects of u-hCG seen in previous studies can be attributed to hCG and provide proof of concept for the use of r-hCG as a prophylactic and a therapeutic agent against early and late stages of breast cancer.

161 Recombinant Human Chorionic Gonadotropin (r-hCG) Significantly Reduces Primary Tumor Cell Proliferation in Patients with Breast Cancer.

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The life-time risk of breast cancer in women is decreased by full-term pregnancy at a young age. The mechanism mediating this protection has been linked to increases in placental human chorionic gonadotropin (hCG) during pregnancy. In addition to preventing tumor initiation, hCG has been shown to inhibit tumor progression in rats by increasing synthesis of the tumor suppressing growth factor, inhibin. The aim of this pilot study was to investigate the effect of recombinant (r-) hCG on tumor cell proliferation in post-menopausal patients with breast cancer.

In this double-blind, placebo-controlled study, 25 post-menopausal women with primary operable breast cancer (T1-T3) received on alternate days for 2 weeks intramuscular injections of either 500 µg r-hCG (Serono; n=20) or placebo (n=5). Core biopsies were collected on day 0 and surgery (mastectomy or lumpectomy) was performed on day 15. The initial biopsy and surgically removed tumor were evaluated to determine the proliferative index, percentage of cells positive for estrogen (ER) and progesterone receptors (PgR), and inhibin immunoreactivity. Samples from three patients were inadequate for immunocytochemistry, leaving evaluable data for 22 patients (18 r-hCG; 4 placebo). The proliferative index, or percentage of cells immunocytochemically positive for Ki67, was reduced from 19.5±13.9 to 4.0±3.8% in r-hCG-treated patients ($p<0.00006$), but in placebo treated patients the proliferative index of the initial biopsy (11.1±7.5) was unchanged in the surgically removed tumor (11.9±8.1) ($p=0.374$). Expression of ER and PgR were decreased, and b-inhibin expression was significantly increased following hormone treatment. These changes were not observed in the placebo group. Hormonal profiles remained characteristic of post-menopausal women. The hormonal treatment was well tolerated and no local or systemic side effects were seen at any time.

In conclusion, r-hCG has an anti-proliferative effect on primary breast cancer tissue and is well tolerated in postmenopausal patients with primary breast cancer.

162 Efficacy of Tamoxifen Following Arimidex™ (Anastrozole) as First-Line Treatment for Advanced Breast Cancer (ABC) in Postmenopausal (PM) Women.

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Introduction: The non-steroidal aromatase inhibitor 'Arimidex' (anastrozole) (ADX) has shown efficacy and tolerability advantages compared with tamoxifen (TAM) as first-line treatment of PM women with ABC. A previous report of a combined analysis of two international randomized, double-blind trials with n=1021 patients (pts) showed that among pts with hormone receptors defined as positive, ADX yielded a significant prolongation of TTP compared with TAM (median 10.7 and 6.4 months, 2P=0.022). For this evaluation median follow-up was 18.2 months (Buzdar et al. ASCO 2000 P154a, Abs 609D).

Methods: In order to assess the efficacy of ADX and TAM as a second-line therapy after the other, the subsequent unblinded treatment was recorded and evaluated.

Results: Of 511 pts who were initially randomized to ADX, 139 received TAM as second therapy. Preliminary data from 98 of these pts showed that 56 pts had a clinical benefit (CR+PR+SD≥24 weeks) while 12 pts had an OR (CR+PR). Of the 510 pts initially randomized to TAM, 140 received ADX as second therapy. Preliminary data from 61 of these pts showed that 41 pts had a clinical benefit and 8 pts had an OR (CR+PR). Of the patients who showed a clinical benefit to either second-line therapy, 50% or more maintained this benefit for a period of 12 months or longer.

Conclusion: Previous studies have shown ADX is effective when given after TAM. These results indicate that TAM is effective after ADX, and add further support to the use of ADX as a first-line treatment in this patient population. Further information will be provided from a randomized trial currently being carried out by the SAKK group assessing cross-over treatment of ADX and TAM.

163 SCH 57058 Is a Selective Estrogen Receptor Modulator (SERM) without Uterotrophic Effects Compared with Either Tamoxifen or Raloxifene.

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Antiestrogens targeted at the treatment and/or prevention of breast cancer should have negligible agonist effects on gynaecological tissues, especially the uterus. SCH 57068 is an orally active non-steroidal antiestrogen which is a potent antagonist of estrogen receptor (ER) function and is devoid of any intrinsic estrogenic activity in ER+ve breast cancer cells. To assess the biological effects of SCH 57068 on the uterus, we compared the relative estrogenic and antiestrogenic activities with those of tamoxifen and raloxifene in the immature rat uterotrophic assay. SCH 57068, tamoxifen or raloxifene were administered by oral gavage over a 4-fold log concentration range (0.01, 0.1, 1.0, 10.0 mg/kg) for a total of 5 days either alone or together with estradiol (E2). Uterine weights were measured and corrected for body weight (bw), and tissues fixed for subsequent analysis of epithelial height and cell proliferation (Ki-67). Tamoxifen given alone stimulated uterine weight up to 3-fold; mean weight at 10 mg/kg dose 151.5±3.1 (±SEM) vs. 57.1±2.9 mg/100g bw vehicle control ($p=0.002$). Raloxifene showed significantly less agonist activity than tamoxifen, although at both 1 and 10 mg/kg dose uterine weights were significantly greater ($p<0.01$) than vehicle control (76.2±2.3 and 90.1±1.2, respectively). In contrast, SCH 57068 demonstrated no agonist effect and uterine weights were identical to vehicle control (60.3±3.5 at 10 mg/kg dose). All three antiestrogens antagonised E2-stimulated uterine weight in a dose-dependent manner; at the maximal 10 mg/kg dose uterine weights (mg/100g bw) were 141.8±5.3 (E2 + tamoxifen), 79.0±1.4 (E2 + raloxifene), 53.6±3.0 (E2 + SCH 57068) compared with 219.5±14.2 for E2 alone control. As an antiestrogen SCH 57068 was significantly more potent than raloxifene, even at the lower 0.1-0.01 mg/kg dose range. These data indicate that SCH 57068 is a pure antiestrogen in the gynaecological tract and suggest that SCH57068 would be an ideal endometrial protection agent for postmenopausal women.

164 The Effect of Anastrozole (Arimidex™) on Serum Lipids – Data from a Randomized Comparison of Anastrozole (AN) Vs Tamoxifen (TAM) in Postmenopausal (PM) Women with Advanced Breast Cancer (ABC).

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Anastrozole is a potent and selective non-steroidal aromatase inhibitor, which reduces estradiol levels in PM women to near undetectable values. A combined analysis of two trials in PM women with ABC has shown AN to have efficacy advantages (time to progression) over TAM in ER+ve patients (Buzdar et al. ASCO 2000 P154a, Abs 609D). The impact of AN and TAM on blood lipids was also monitored during these trials. Blood samples for lipid assessment [total cholesterol (TC), triglycerides, HDL, LDL, apoprotein A, apoprotein B, and lipoprotein a] were taken at baseline, 84, and 108 weeks. Preliminary blood lipid results are shown below. No major differences were seen for the other lipid endpoints.

	Baseline value [mmol/l (n)]		Mean change at 84 weeks (n)		Mean change at 108 weeks (n)	
Blood lipid	AN	TAM	AN	TAM	AN	TAM
TC	5.8 (476)	5.9 (511)	+0.3 (67)	-0.6 (55)	+0.3 (24)	-0.2 (31)
HDL	2.4 (306)	3.7 (304)	-1.0 (38)	-2.2 (36)	-2.1 (17)	-2.2 (23)
LDL	3.7 (306)	3.8 (304)	+0.2 (38)	-0.9 (36)	+0.1 (17)	-0.5 (23)

The effects of TAM were similar to that reported previously, but no major differences from effects of AN were observed. Despite its potent estradiol lowering properties, AN had no clinically detrimental effects upon blood lipids. These data suggest that clinical effects of AN due to any changes in lipid profiles are very unlikely.

165 Clinical and Endocrine Data for Goserelin (Zoladex) Plus Anastrozole (Arimidex) as Second Line Endocrine Therapy in Premenopausal Women with Advanced Breast Cancer.

Forward DP, Cheung K-L, Jackson L, Robertson JFR. Professorial Department of Surgery, Nottingham City Hospital, Nottingham, United Kingdom.

Fifteen premenopausal women with either metastatic breast cancer (12) or locally advanced primary breast cancer (3) were treated with a combination of a gonadotropin releasing hormone agonist, goserelin, (Zoladex (Z)) and a selective aromatase inhibitor, anastrozole (A). All had previously been treated with Z and tamoxifen (T). Ten patients achieved objective response or durable static disease (OR/SD) at six months with a median duration of remission of 14 months (6 - 38 months). Three patients remain in OR/SD but have yet to achieve six months on therapy. Two patients progressed before the six month assessment (median duration 2.5 months).

The introduction Z+T resulted in an 89% reduction in oestradiol (E2) levels compared to pre-treatment - E2 concentration pre Z+T 224 pmol/L and after 6 months 24 pmol/L. Substitution of T by A on progression resulted in a further 76% fall in serum E2 levels associated with clinical regression - E2 concentration on Z+T was 24 pmol/L and after 3 and 6 months on Z+A was 6 and 5 pmol/L respectively.

This study shows that Z+A induces therapeutic remission in patients with advanced breast cancer who have progressed on Z+T. The serum E2 levels show a further reduction when anastrozole (in combination with Z) is substituted for tamoxifen (in combination with Z) to levels seen in post-menopausal patients receiving anastrozole.

166 Pre-Clinical Pharmacology Profile of a New Selective Estrogen Receptor Modulator (SERM), ERA-923, for the Treatment of ER Positive Breast Cancer.

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While tamoxifen is effective in the control and prevention of ER-positive (ER+) breast cancer, more effective therapy with fewer side-effects is sought. In particular, women with ER+ metastatic breast cancer, have, or rapidly acquire, resistance to tamoxifen.

ERA-923, a 2-phenyl indole, is a new SERM. The compound inhibits estrogen binding to the ER (IC_{50} = 45 nM) and inhibits estrogen-regulated estrogen response element- or C3-promoter activity (IC_{50} = 6.7 nM and 3.8 nM, respectively), but has no agonistic effects when given alone. In ER+ human MCF-7 cells, ERA-923 inhibits estrogen-stimulated growth (IC_{50} = 0.7 nM) associated with cytostasis and G_0/G_1-cell cycle arrest. An MCF-7 variant that is inherently resistant to tamoxifen (10-fold) or 4-OH tamoxifen (>1000-fold) retains complete sensitivity to ERA-923. Partial sensitivity to ERA-923 is also found in MCF-7 variants that have acquired profound resistance to tamoxifen or 4-OH tamoxifen. In tumor-bearing animals, ERA-923 (3-10 mg/kg/day given orally (PO)), inhibits estrogen-stimulated growth in tumors derived from MCF-7, human endometrial EnCa-101, or human ovarian BG-1 cells, including an MCF-7-variant that is inherently resistant to tamoxifen. Unlike tamoxifen, no uterotrophic effects are observed when ERA-923 is given alone (in the absence of estrogen) to rats or mice. Consistent with these data, ERA-923, when given alone, does not stimulate the growth of EnCa-101 tumors, whereas tamoxifen stimulates tumor growth. ERA-923 given at 3 mg/kg/day (PO) preserves bone mineral density and lowers total cholesterol in rat models. These data suggest that ERA-923 may have a superior efficacy and safety profile compared to tamoxifen in women with ER+ breast cancer, particularly in those patients with tamoxifen-resistant disease. Based on these and other results, a Phase I trial with ERA-923 given to healthy women was done. A Phase II trial in women with metastatic breast cancer is in progress.

167 Promising Activity and Safety of Exemestane (E) as First-Line Hormonal Therapy (HT) in Metastatic Breast Cancer (MBC) Patients (pts): Final Results of an EORTC Randomised Phase II Trial.

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E, an irreversible steroidal aromatase inactivator, has superior efficacy to megestrol acetate in MBC pts who have failed tamoxifen (T) (Kaufman JCO 18: 1807,2000). Adjuvant (adj) T is now routine for hormone receptor (HR) + breast cancer, thus alternative 1st line HTs are needed for MBC. This EORTC phase II randomised postmenopausal MBC pts to 1st line E 25mg/day or T 20mg/day; endpoints were activity and safety. Eligibility criteria have been described (Paridaens Proc ASCO 19:83a,2000). The phase II closed 31/05/00 with 122 pts. Characteristics are available for 107 to date (E 57, T 50). For E and T respectively, median (med) age was 62 (range 37-85) and 63 (46-87); ECOG performance status of 0/1/2 was 20/29/8 and 17/26/7; med disease-free interval was 3.5y and 4.6y. At least 1 HR was + in 89% and 86% of E, T pts. The dominant site was: soft tissue 10 E, 4 T; bone 15 E, 16 T; visceral 32 E, 30 T pts. Ten pts each had adj T; med time off T was E 3.4y (1.6-7.8), T 3.7y (1.1-14.5). No pts discontinued due to toxicity (tox). There were no grade 4 (NCIC) tox; the most common non-haematologic tox (NCIC) for assessable pts (53 E, 49 T) are:

Grade 2/3 tox	% E	% T	Grade 2/3 tox	% E	% T
Anorexia	4	8	Edema	2	8
Nausea	4	8	Dyspnea	11	10
Constipation	4	4	Hot flushes	4	12
Weight gain	4	8	Sweating	0	10
Weight loss	4	0	Pain	19	31
Fatigue	8	12	Anxiety/insomnia	8	4
Skin	8	0	Fever/infection	11	6
Phlebitis	0	2	Myalgia/arthralgia	2	2

For E and T, preliminary data was: response rate 42%, 16%; med time to progression (TTP) 8.9, 5.2 months (m) (Paridaens Proc ASCO 19:83a, 2000). Updated tox and a final peer-reviewed efficacy analysis will be presented. Based on promising activity of E, a phase III extension was initiated with 80% power to detect an increase from 8 to 10 m in TTP with E (n=768, 632 events needed).

168 Endometrial Histopathology in 700 Patients (pts) Treated with Tamoxifen (Tx) for Breast Cancer.

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Objective: Evaluation of endometrial histopathologic findings from 700 pts treated with Tx for breast cancer from two medical centers (USA and France).

Methods: Retrospective review of data including histologic slides from 134 hysterectomies and 566 endometrial biopsies from Tx-treated pts who presented with abnormal vaginal bleeding and/or abnormal sonograms ; analysis of histologic characteristics ; recording of duration of Tx therapy when available and correlation with endometrial pathology.

Results: The only statistically significant difference between the data from the USA and France was the number of hysterectomies, almost double in France (27 % vs 13,7 %). Non-pathologic endometria were 61,14 % (inactive/atrophic 46 %, functional 15,14 %). Pathologic changes were found in 39,86 % cases, of which polyps were 23,14 %, glandular hyperplasia 8 % metaplasia 3 %; endometrial cancer 4,71 % (33 cases). Nine cancers were well differentiated endometrioid adenocarcinomas, 24 moderately or poorly differentiated, of which 13 with non-endometrioid components (serous, clear cell, MMMT). Fifteen cancers were found in endometrial polyps, 12 were invasive to the myometrium and 4 to blood vessels. The average age of all pts was 60,91 years and of the cancer pts 69,26 years. The shortest average duration of Tx therapy (2,5 yrs) was found in pts with inactive/atrophic endometria, the longest (6,8 yrs) in pts with endometrial cancer. Pts with endometrial polyps and cancer presented more often with abnormal vaginal bleeding than those with inactive/atrophic endometrium.

Conclusions: Most Tx-treated pts had no pathologic endometrial changes. Endometrial polyps, hyperplasia and metaplasia, consistent with an estrogen-agonist effect of Tx were found in roughly one-third of all pts. The endometrial cancers were often high grade and invasive tumors. Pts with endometrial pathology were more often symptomatic than pts with inactive/atrophic endometria.

169 Intra-Tumoral Variation in Pathological Assessments of Apoptosis and Proliferation in Breast Cancer Biopsies.

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There is increasing interest in the optimisation of treatment in early breast cancer by the use of predictive factors. This has led to studies examining changes in treatment-related biological markers. However little is known about the influence of tumour heterogeneity or a previous surgical biopsy upon such markers. We have therefore studied proliferation, apoptosis and hormonal-regulated proteins (possible surrogate markers of endocrine sensitivity) in primary post-menopausal breast cancers. Paired pathological specimens taken without any intervening therapeutic intervention were studied: 13 patients with both core biopsies and surgical excision taken at the same time, and 17 women with a diagnostic core taken three weeks before definitive surgical excision. Proliferation was assessed by counting the percentage of positive cells using the MIB-1 antibody, and apoptosis as the percentage of positive cells using a TUNEL staining method. Bcl-2 expression was assessed with the DAKO 124 antibody.

There was no change in proportion of bcl-2 positive cells, although 4/21 (19%) tumours had a reduction in intensity in the specimen removed three weeks later. No significant differences were evident between the median apoptotic and proliferative scores in the two tumour samples for either group of patients, although there was a trend for apoptosis to be lower in the second sample ($p < 0.09$). However, there was marked variation between specimens from individual tumours with only 20% and 11% of patients having < 10% difference in the MIB-1 & TUNEL assays respectively, with respectively 27% and 63% of patients having > 50% difference.

These results suggest that although multiple samples of the same tumour do not display significant differences in apoptosis and proliferation, the large variation in values will detract from the clinical utility of these parameters.

170 Exemestane as Neoadjuvant Treatment for Locally Advanced Breast Cancer: Endocrinologic and Clinical Endpoints.

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13 patients, mean age 77.3 (range 58-88 years), with large operable (n=12) or locally advanced (n=1) breast cancer with ER rich (>80 histoscore) breast cancer have been treated in a phase II trial of neoadjuvant exemestane. Following an 18 hour infusion of ^{3}H-androstenedione and ^{14}C-estrone, all patients had an open biopsy of tumour and surrounding non-malignant breast. Patients were then treated with exemestane (25mg/day) for 3 months. Tumor response was assessed by monthly clinical examination and ultrasound; additionally mammography was performed prior to and after 3 months. Immediately before definitive surgery at 3 months, patients had a second dual isotope infusion and further tumour and non malignant breast tissue sampled. Radioactivity was measured in purified estrogen fractions allowing assessment of changes in intratumoral aromatase and oestrogen uptake. Similar measurements were made in plasma samples taken at the same time, providing estimates of peripheral aromatase. The median percentage reduction in tumour was 85.5% on clinical evaluation, 82.5% using ultrasound and 84% using mammography. Using UICC criteria 10 out of 12 subjects had a 50% reduction in bidimensional clinical measurement, 1 subject had a minor response and 1 subject showed no change. Changes in ultrasound volume (USS) are shown in table.

Response Status	Partial Response	Minor Response	No Change	Progressive Disease
UICC	10	1	1	0
USS volume	10	2	0	0

Exemestane produces a profound reduction in aromatase in the periphery and in both non malignant and malignant tissue from all patients. Exemestane dramatically reduces peripheral and intratumoral estrogen synthesis and these changes translate into significant tumour shrinkage in the neoadjuvant setting.

171 Letrozole Is Highly Effective in Patients with Soft Tissue Metastases.

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Introduction: The nonsteroidal aromatase inhibitor Letrozole 2.5 mg has previously shown superiority over megestrol acetate (MA) as second-line therapy in postmenopausal women with advanced breast cancer (ABC). The purpose of this paper is to analyze the efficacy of letrozole and MA with respect to response (OR = CR+PR), duration of response (DR) and overall survival (TTD) for patients with predominantly soft tissue metastases using machine learning techniques.

Methods: 150 ABC-patients with soft tissue metastases [Mean age: 66.2; disease free interval ≥ 24 mths: 61.3%; no previous chemotherapy: 73.3%; objective response to prior therapeutic antiestrogen: 25.3%; performance status WHO grade 0: 66.0%] were randomly assigned to receive letrozole 2.5 mg (=L25; n =44), letrozole 0.5 mg (=L05; n = 57), or MA 160 mg (=MA; n = 49) once daily in a double-blind, multinational, peer reviewed trial until progression of disease or any other reason requiring discontinuation of study treatment.

Results: L25 is significantly more effective than MA [1.3 times more clinical benefit (p=.011) resp. 1.4 times more objective response (p=.009) and 1.6 times longer mean duration of OR (p=.003)]. If DFI > 24 mths then CB = 79.2% (OR=70.8%) for L25. If DFI > 24 mths and both hormone receptors positive then OR and CB > 80%. The most important prognostic factors for OR are (C4.5-algorithm): Treatment, disease free interval, age (OR is increasing with age), receptor status, previous chemotherapy (negative impact). Logistic regression delivers only treatment as prognostic factor with 64.4% correct classification.

	CR	PR	SD	PD	OR	CB	DR (Mean)	DR (Median)	TTD (OR)
L25	22.7%	31.8%	13.6%	31.8%	54.5%	68.2%	922	735	1269
L05	10.5%	14.0%	17.5%	52.6%	24.6%	40.4%	926	981	1316
MA	14.3%	24.5%	14.3%	38.8%	38.8%	51.2%	592	563	1074

*OR = objective response; CB = clinical benefit = CR + PR + SD >6 mths); DR = mean (median) duration of OR (days); TTD(OR) = mean survival of patients with OR (days).

Conclusion: Letrozole 2.5mg is highly active in ABC-patients with soft tissue metastases and significantly more effective than MA. There is a significant dose response effect between letrozole 0.5mg and 2.5mg in favour of letrozole 2.5mg.

172 A Comparison of the Single-Dose Pharmacokinetics (PK) of 'Faslodex' (Fulvestrant) 250 mg When Given as Either a One x 5-ml Intra-Muscular (i.m.) Injection or Two x 2.5-ml Injections in Postmenopausal (PM) Women with Advanced Breast Cancer (ABC).

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Faslodex (ICI 182,780)(FAS) is a novel estrogen receptor downregulator currently in clinical trials versus anastrozole ('Arimidex') and tamoxifen ('Nolvadex') in PM women with ABC, where the dose tested is FAS 250 mg i.m. once monthly. In North American based FAS trials, differences in clinical practice preferences led to FAS 250 mg being given as 2 x 2.5-ml injections as opposed to 1 x 5-ml dose in Europe/ Rest of World based trials. Given the differences in dose administration within the FAS breast cancer trial programme, it was therefore important to compare the PK of FAS when given as either 1 x 5-ml dose or 2 x 2.5-ml dose.

Here we report the PK findings of an open, randomized multicenter, parallel-group trial in PM women with ABC. 38 patients received a single dose of FAS, either as 1 x 5-ml injection (n=20) or 2 x 2.5-ml injections (n=18). Blood samples for PK analysis were taken at various time points up to 28 days after treatment. Tolerability assessments were also made. PK parameters included AUC 0-28 days, C 28 days, C max and t max. Safety follow-up continued until 8 weeks after the injection was given.

The geometric mean AUC 0-28 days blood levels were 106.8 ng/ml/day and 105.5 ng/ml/day for 1 x 5 ml and 2 x 2.5 ml respectively. The ratio of the geometric means of 1.01 (95% CI 0.68-1.51) showed there was no significant difference in AUC between the two dose regimens (p=0.94). The geometric means of C 28 days, and C max and the median of t max were similar in both treatment groups. Both treatment regimens were well tolerated, with there being no major differences in adverse events. The data from this study were in line with AUC 0-28 days data from an earlier study involving postmenopausal women with primary breast cancer, where based on 22 women receiving FAS 250 mg (1 x 5 ml), the geometric mean AUC 0-28 day was 116.5 ng/ml/day.

In conclusion, there was no significant difference in PK and adverse events between 1 x 5-ml injection and 2 x 2.5-ml injections of FAS. Based on these PK findings, the dosing regimen employed with FAS in the clinical setting, would not be expected to impact on the clinical outcome indicating that the 250 mg dose of FAS may be administered as either 1 x 5-ml injection or 2 x 2.5-ml injections. Additionally, the single-dose PK findings in two trials using FAS 250 mg (1 x 5-ml) demonstrate the consistency of FAS PK between trials.

173 Anastrozole Vs Tamoxifen in Hormonodependent Advanced Breast Cancer. A Phase II Randomized Trial.

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BACKGROUND: In this report we present the results of a prospective, double blind randomized triaal comparing a new non-steroidal aromatase inhibitor (Anastrozole) vs Tamoxifen in a group of postmenopausal patients with hormonodependant advanced breast cancer. Main end points were response rates, toxicity profile analysis, median time to progression and overall survival.

SUBJECTS AND METHODS: To be included in the trial, patients have to display the following characteristics: 1. Histopathological diagnosis of advanced breast cancer 2. Measurable/evaluable lessions 3. ECOG PS 0-2 4. Adequate organ functions 5. Positive estrogen receptors 6. No previous therapy for advanced disease 7. No previous hormonal adjuvant therapy 8. Postmenopausal status 9. Signed informed consent. Elegible patients were randomized to receive either Anastrozole 1 mg/or/daily or Tamoxifen 40 mgs/or/daily. After 3 months therapy was started, responses were assesed following WHO/ECOG guidelines. Curves were constructed according to the Kapla & Meier methodology and were compared by means of log-rank test.

RESULTS: From May 1997 to November 1999 a total of 238 patients were included in the study (121 Anastrozole arm/ 117 Tamoxifen arm). Overall response (CR+PR) was 34 % and 27 % respectively. Median Time to Progression was 10.6 months for Anastrozole and 5.3 months for Tamoxifen, with a higher risk of tumoural progression in the Tamoxifen group as indicated by the hazard ratio analysis (0.77, 95 % CI = 0.56 - 0.91. p< .05). By the cut-off data, 61 % of the patients in the Anastrozole group have died while 92 % of the patients died in the Tamoxifen group. The hazard ratio for death analysis was 0.63, 95 % CI = 0.51 - 0.89, p< .05.

Therapy in both therapeutic arms was well tolerated with a low incidence of undesirable effects.

CONCLUSIONS: Anastrozole is an efficent and well tolerated treatment for posmenopausal patients with hormonal dependant advanced breast cancer. Our data suggest is a good alternativeto Tamoxifen as firts line therapy in such patient's group.

174 Letrozole and Megestrole Acetate in Patients with Advanced Breast Cancer (ABC) Resistant to Tamoxifen (TAM).

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Introduction: The nonsteroidal aromatase inhibitor Letrozole 2.5 mg has previously shown superiority over megestrol acetate as second-line therapy in postmenopausal women with advanced breast cancer (ABC). The purpose of this paper is to analyze the efficacy of letrozole 2.5mg (L25), letrozole 0.5mg (L05) and megestrole acetate (MA) with respect to objective response rate (RR), duration of response (DR) and overall survival (TTD) for patients resistant to TAM.

Methods: 552 ABC-patients [Mean age: 64;1 anatomical site of metastatic diseases: 57.6%; response to prior therapeutic antiestrogen: CR+PR: 20.5%; NC or response unknown but antiestrogen given for at least 6 months (=NC*): 32.2%; PD or response unknown but antiestrogen given for less than 6 months (=PD*): 11.4%; not applicable (adjuvant therapy, etc): 35.9%] were randomly assigned to receive L25 (n =174), L05 (n = 188), or MA (n = 190) daily in a double-blind, multinational, peer reviewed trial.

Results: In patients treated with L25, response and duration of response were similar for patients resistant to TAM and patients showing an objective response to TAM, respectively. Furthermore, there is a trend towards higher response in patients resistant to TAM compared to patients with NC or response unknown but antiestrogen given for at least 6 months. L25 is superior to L05 and MA with respect to response (p=.015) and duration of response (p = .011) in patients resistant to TAM.

*	RR(CR+PR)	RR(NC*)	RR(PD*)	DR(PD*)	DR(CR+PR)	TTD(PD*)
L25	42.4%	26.2%	38.1%	1110	735	1109
L05	20.0%	18.3%	6.7%	all censored	851	604
MA	12.5%	22.8%	14.8%	257	548	633

* RR(X) = Objective response to L05, L25 or MA of pts with response X to prior antiestrogens (1st line therapy); DR(X) = median duration of objective response to L05, L25 or MA of pts with response X to prior antiestrogens (days). TTD(X) = mean survival time of pts with objective response to L05, L25 or MA and response X to prior antiestrogens.

Conclusion: TAM-resistant and TAM-responding ABC-pts have similar treatment outcome with letrozole 2.5mg. In TAM-resistant ABC-pts, Letrozole 2.5mg shows a significantly higher RR, longer duration of response and longer survival time compared to MA. Some patients respond to letrozole but not to TAM and vice versa.

175 Adenovirus Mediated Expression of E2F-1 in Combination with Paclitaxel Has a Synergistic Effect on Breast Cancer Growth Inhibition.

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The transcription factor E2F-1 has been shown to cause apoptotic cell death in breast cancer cells. Previous work from our laboratory has shown that adenovirus mediated overexpression of E2F-1 can increase the sensitivity of breast cancer cells to chemotherapeutics. In this study, we evaluated the efficacy of recombinant adenovirus expressing E2F-1 (Ad5E2F-1) in combination with paclitaxel using a two dimensional isobologram methodology. **Methods:** MDA-MB-361 breast cancer cells were plated at $2x10^3$ cells per well into 96 well plates and then infected with Ad5E2F-1 at MOIs of 0, 10, 50, 100, 500, and 1000. Twenty-four hours after the infection, various doses of paclitaxel (0, 10, 100, 500nM, and 5μM) were added to the cells. Forty-eight hours after adding paclitaxel, cell viability was assessed by XTT assay. Data were analyzed using a two dimensional isobologram method at both 50% and 80% growth inhibition (ID_{50} and ID_{80}) levels to evaluate the presence of synergistic, additive, or antagonistic efficacy of Ad5E2F-1 and paclitaxel. We also subjected the cells to FACS analysis after the treatment to assess for apoptosis. **Results:** We observed marked synergistic growth inhibitory effects with Ad5E2F-1 and low doses of paclitaxel and additive effects with Ad5E2F-1 and moderate doses of paclitaxel. Ad5E2F-1 caused apoptotic cell death alone and we noted increases in the number of apoptotic cell death at all doses of paclitaxel. **Conclusions:** Adenovirus mediated expression of E2F-1 acts synergistically in combination with low dose paclitaxel to inhibit breast cancer cell growth. This appears to result from induction of apoptosis. Combining gene therapy and chemotherapy may allow for lower doses of chemotherapeutic agents, thus reducing adverse effects of chemotherapy while still achieving a high rate of cell killing.

176 Mechanisms of HET/SAF-B Mediated Repression of Estrogen Receptor's Transcriptional Activity.

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Defining the role of estrogen receptor and its interacting proteins is critical to understanding those cellular pathways involved in the progression from healthy tissue to cancer. We have cloned a gene for a nuclear matrix protein HET/SAF-B (Hsp27 ERE TATA binding protein/Scaffold attachment factor-B) that interacts with the estrogen receptor (ER) as a co-repressor (Mol. Endocrinol. 2000; 14:369-381). Data from clinical breast cancer samples has indicated that there is a high loss of heterozygosity at the HET/SAF-B locus on chromosome 19p13. We have identified mutations in HET/SAF-B in some of these samples. This suggests that HET/SAF-B functions as a tumor suppressor gene in breast cancer.

We are interested in defining the biochemical mechanisms of how HET/SAF-B functions. Our hypothesis is that HET/SAF-B represses ER mediated transcriptional activation by recruiting a chromatin-remodeling protein complex to ER target promoters and by interacting with the transcriptional machinery. We are currently identifying the domains in HET/SAF-B responsible for interaction with ERα and for transcriptional repression. This will then allow us to investigate if and how the mutations in HET/SAF-B identified in tumor samples interfere with HET/SAF-B's function as an ERα co-repressor.

177 Defective Tumour Necrosis Factor-a (TNF-a) Production and Impaired TNF-a - Induced ICAM-1 Expression in BRCA1 Mutation Carriers.

Budinsky AC, Wagner TMU, Kubista M, Wolfram RM, Kubista E, Brodowica T, Zielinski CC.

Background: Tumour necrosis factor-a (TNF-a) is a potent cytokine secreted primarily by activated cells from the monocyte/macrophage lineage and induces of apoptosis, necrosis, activation of lytic effector cells and upregulates the expression of intercellular adhesion molecule-1 (ICAM-1). Previous studies from our laboratory have indicated impaired production of TNF-a by monocytes as well as decreased expression of ICAM-1 on monocytes derived from patients with various stages of breast cancer.
Methods: Spontaneous as well as LPS-induced production of TNF-a by as well as expression of ICAM-1 on monocytes derived from healthy females with germline mutations of BRCA1 and from healthy age-matched control females were assessed.
Results: Monocytes derived from healthy women with various germline mutations of BRCA1 had significantly decreased spontaneous ($p=0.03$) and lipopolysaccharide (LPS)-induced ($p<0.001$) production of TNF-a, as compared to monocytes derived from healthy age-matched control females, whereas no difference in LPS- or TNF-a - induced production of interleukin-6 (IL-6) was found. Unstimulated monocytes derived from healthy women with germline mutations of BRCA1 and from healthy control women had similar expression of ICAM-1, but stimulation with cytokines TNF-a and/or interleukin-1 led to a significant increase of ICAM-1 expression on monocytes derived from control females only, but not from BRCA1 germline mutation carriers ($p<0.001$).
Conclusion: We conclude that the presence of germline mutations of BRCA1 was associated with a deficiency in production of TNF-a and of TNF-a - induced ICAM-1 expression on peripheral blood monocytes thus constituting an additional biologic variable apart from BRCA1 possibly contributing to the development of breast cancer.

178 Cyclins, CDK-Inhibitors and Retinoblastoma Protein Aberrations in Breast Cancer.

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The cell cycle machinery is regulated by cyclin dependent kinases and sets of activating and inhibitory proteins. The G1-S control mechanism is often deregulated in tumors supposedly leading to increased kinase activity, phosphorylation of substrates and subsequent S-phase entrance. To understand the interplay between the expression of G1-S regulatory proteins, tumor proliferation and tumor aggressiveness we have in detail characterized the type of G1-S transition aberration, in vivo phosphorylation of the retinoblastoma protein (pRb), proliferation and the estrogen receptor content in 104 primary breast cancer samples. The cyclin E dependent kinase activity (cyclin Ekinase) was further determined in a subgroup of 59 primary breast cancers, using an H1-kinase assay. In the estrogen receptor positive tumors there were significant associations between cyclin D1, cyclin E, cyclin Ekinase, pRb phosphorylation and proliferation with an expected behavior regarding activation of cyclins, phosphorylation of a key substrate and affected proliferation. This is in contrast to estrogen receptor negative tumors that predominantly consisted of cyclin D1 low tumors with either high or low cyclin E protein contents and pRb inactivated tumors. These tumors showed inverse associations between cyclin E and D1, lack of associations between pRb phosphorylation and proliferation despite strong association between cyclin E, cyclin Ekinase and proliferation. p53 mutations were predominantly observed in tumors with high cyclin E and low cyclin D1 expression whereas tumors with a combined overexpression of cyclin E and D1 were exclusively p53 normal.
Based on studies of cell lines transfected with various vectors and patterns of aberrations in proteins regulating the G1/S transition in primary breast cancer samples we therefore suggest that breast cancer can be divided in separate groups that follow different pathways to obtain unrestrained cell growth. These groups also show partly contrasting malignant behavior and clinical outcome suggesting that the type of aberration in the G1/S transition is not only linked to proliferation but also to other properties associated with tumor aggressiveness.

179 Analysis of Differential Downstream Signaling between Breast Cancer Cell Lines Overexpressing Epidermal Growth Factor Receptor and ErbB2.

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Our laboratory has previously shown that downregulation of the adaptor protein Grb2 via liposomal Grb2 antisense oligonucleotides (L-Grb2 AS) resulted in decreased cell proliferation in breast cancer cell lines overexpressing the ErbB family members epidermal growth factor receptor (EGFR) and ErbB2. In addition, Grb2 downregulation unexpectedly resulted in differential decreases in downstream signaling proteins. In EGFR overexpressing cells, L-Grb2 AS decreased activity of ERK but not Akt/PKB; conversely, in ErbB2 overexpressing cells, Akt/PKB but not ERK was inhibited, suggesting that signaling pathways regulating cell growth differ between EGFR and ErbB2 overexpressing breast cancer cells.
Based on these results, we propose that differential downstream responses between EGFR and ErbB2 overexpressing breast cancer cells are due to differences in Grb2 complex formation and/or differences in signaling intermediates. Thus, we are examining (1) Grb2 coprecipitation with proteins implicated in ERK and Akt/PKB pathways (SOS-1, Shc, PI3K, Gab1), and (2) activity of Ras, a well-characterized intermediate in the Grb2-ERK pathway and a potential signaling protein in the Grb2-Akt/PKB pathway, via Ras pull-out assay using GST-RBD Raf. Analyzed breast cancer cell lines include naturally overexpressing Erb family members Sk-BR-3 (ErbB2) and MDA-MB-468 (EGFR). To minimize effects of genetic differences between cells, ErbB family-transfected MCF-7 cells (MCF-7/HER2, MCF-7/EGFR) were also examined. Preliminary results in untreated naturally overexpressing EGFR and ErbB2 cell lines show no clear difference in Grb2 complex formation with the proteins examined, suggesting that differences in signaling between EGFR and ErbB2 overexpressing cells are not apparent until L-Grb2 AS treatment and/or are downstream of Grb2 complex formation.
In Ras pull-out assays, preliminary results in untreated cells show that Ras activity is greater in ErbB2 and EGF-stimulated EGFR overexpressing cells compared to MCF-7, indicating that Ras is activated upon activation of both ErbB family members. These results suggest that Ras may be involved in both EGFR-ERK and ErbB2-Akt pathways. Treatment of these cell lines with L-Grb2 AS followed by examination of Ras activity are underway.

180 HER-2/neu/ErbB-2 Status by Immunohistochemistry and FISH: Clonality and Progression with Recurrence and Metastases.

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INTRODUCTION: Treatment of recurrent or metastatic breast cancer patients with Herceptin requires evidence of ErbB-2 overexpression by analysis of the primary tumor. Limited data are available regarding the correlation between the HER-2/neu/ErbB-2 status of the primary and the recurrent or metastatic disease. Preliminary data from ongoing Herceptin trials suggest that only a portion of patients with metastatic disease have responded to Herceptin using the Dako HercepTest as the entry criteria. Marked genetic heterogeneity of HER-2 gene copy number has been reported in primary ductal carcinomas, suggesting clonal heterogeneity. Increased amplification/overexpression of ErbB-2 in recurrent disease or metastases may be associated with clonal selection. METHODS: Breast cancers stained with the Dako HercepTest and CB11 immunoassays for ErbB-2, +/- computerized imaging quantitation (ChromaVision technology) on 193 primary breast cancers and corresponding local recurrences (68), lymph node metastases (32), and distant metastases (93) will be compared. FISH assay data reflecting gene amplification levels will also be analyzed. Immunohistochemical and FISH ErbB-2 data will be compared to primary tumor size, grade, nodal status, time to failure (recurrent or metastatic), and survival. RESULTS: Data suggests that up to 25% of recurrences and metastases (n=193) have discordant HER-2 data. CONCLUSIONS: Establishing the degree of concordance between results obtained from primary tumors and relapsing disease may be critical for utilization of anti-HER-2 therapeutics. Supported by PO1 CA44678 and the Evanston Northwestern Healthcare Auxiliary.

201 Analysis of p43 Positive Lymphocytes from Peripheral Blood for Early Detection of Non Palpable Breast Cancer.

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Objective: Regular screening mammographies and increasing knowledge of high-risk groups have resulted in an improvement in the rate of detection of smaller malignant lesions. However, uncertain minimal mammographic features frequently require further costly and often uncomfortable investigation, including repeat radiological controls or surgical procedures, before cancerous lesions can be identified. A reliable diagnostic marker is therefore useful to differentiate between women with early stage breast cancer and women with benign breast lesions.

Study Methods: In a double blind, randomised, multicenter study we evaluated the sensitivity and specificity of the expression of p43-positive lymphocytes as a marker in early stage breast cancer and also investigated its expression on T-cell subpopulations The presence of p43-positive lymphocytes was investigated using the monoclonal antibody CM-H-9 and flow cytometry in 214 women with controversial, non palpable mammographic findings who were undergoing surgical biopsy.

Results: Patients with early breast cancer (n=96) had significantly higher p43-positive cell values (median 3.98 %, range 0.98 to 19.4) than patients with benign lumps (n=118, median 1.22 %, range 0.17 to 3.7) ($p<0.0001$). At a cut-off level of 2 % p43-positive cells a sensitivity of 89.7 % and a specificity of 89.1 % for detection of early breast cancer could be reached. While the median ratio of total CD4+/CD8+ cells was 2.6, a ratio of 1.3 was found for the p43-positive subpopulation ($p<0.001$), thus indicating a significant link between p43 and CD8+ cells.

Conclusions: The determination of p43-positive lymphocytes in peripheral blood could serve as an additional diagnostic tool in patients with controversial mammographic findings and could also reduce the need for cost-intensive and often uncomfortable management of these patients.

202 Refined HER2/neu Diagnosis in Breast Cancer by Consecutive Immunohistochemistry and FISH.

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The therapy of metastatic breast cancer with Herceptin (trastuzumab) is gaining in importance. Since only patients with HER2-positive tumors will benefit by a Herceptin treatment the reliable diagnosis of HER2 is indispensable. Among many diagnostic techniques immunohistochemistry (IHC) and fluorescence in situ hybridisation (FISH) turned out to be practicable. FDA-approved kits are available for both methods. Anyway, both techniques have their advantages but also their pitfalls. IHC is cheap and easy to perform but can be impaired by the pretreatment of the tumor material. In former studies the FISH results correlated better with the clinical outcome of the patients. It is a very reliable but demanding and expensive technique. We compared the results of both methods and established a two step diagnosis in order to combine their advantages for the indication of Herceptin treatment.

The tumors of 142 patients were examined thus far. The HER2 overexpression was determined with the HercepTest kit (DAKO), the amplification of the gene with Ventana's Inform kit. The results of both techniques were consistent for an IHC score of 0, 1+, and 3+ but differed markedly at 2+: In all specimens (44) with 0 and 1+ no gene amplification was detectable. All cases (26) with 3+ were amplified. Of 72 tumors with 2+ we found 50 to be not amplified, ten were moderately amplified (<10 gene copies), and 12 turned out to be highly amplified.

From these results we deduced a two step HER2 diagnosis: first, all tumors are analyzed by IHC. Specimens with a score of 2+ are additionally subjected to FISH. As for all 3+ cases, Herceptin therapy is indicated for the patients with 2+ and >10 gene copies. This strategy seems to be very effective. We found a response in 4 of 8 patients of this subgroup treated with Herceptin/chemotherapy. These findings are in contrast to the clinical trials where the total 2+ group did not benefit significantly by Herceptin treatment.

203 FDG-PET for the Detection on Recurrent or Metastatic Breast Cancer.

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FDG-PET is known to be a non-invasive imaging technique, which is capable of identifying primary tumors and metastases with high sensitivity and accuracy. The aim of this study was to evaluate the diagnostic accuracy of whole-body FDG-PET imaging for the detection of recurrent or metastatic breast cancer after surgery. Whole-body FDG-PET imaging was performed on 27 patients with suspected recurrent breast carcinoma. PET images were evaluated qualitatively for each patient and lesion. FDG-PET scans showed that there were 61 reference sites of malignant or benign lesions in 27 patients. In a patient-based analysis, FDG-PET scans correctly identified 16 of 17 patients with recurrent or metastatic disease and 8 of 10 without recurrence, resulting in a sensitivity, specificity and accuracy of 94%, 80% and 89%, respectively. In a lesion-based analysis, FDG-PET scans correctly identified 46 of 48 lesion sites with recurrent or metastatic disease and 11 of 13 without recurrence. Overall sensitivity, specificity and accuracy for all lesion sites were 96%, 85% and 93%, respectively. FDG-PET scans revealed unsuspected recurrent or metastatic diseases in 8 of 27 (30%) of patients and 11 of 20 (55%) distant metastatic lesions. In 13 patients, treatment was altered by the outcome of the PET scan. We concluded that whole-body FDG-PET scan is a useful diagnostic imaging modality for the detection of recurrent or metastatic breast carcinoma in patients suspected of having recurrent disease after primary surgery.

204 Pilot Study of 0.5 Tesla Dedicated Magnetic Resonance Imaging for Early Detection of Breast Cancer in Young High Risk Women.

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The sensitivity of film-screen mammography in women < age 50 compared to older women is limited by a higher prevalence of increased breast density, faster-growing tumors, or both. High risk women present an especially challenging dilemma. Breast density does not pose a limitation to MRI. Furthermore, theoretical risks of radiation exposure in young women who may harbor DNA repair defects are averted. Although breast MRI studies indicate a high sensitivity, low specificity is a concern. Limited data are available in the high-risk surveillance setting and most published studies have used higher-strength magnets, not practical for most breast centers.

We studied 30 women age 25-49 years with no history of breast cancer who had dense breast tissue and a high quantitative breast cancer risk or known *BRCA1* or *BRCA2* germline mutation. Breast density was prospectively rated using the BIRADS lexicon applying "heterogenously dense" and "extremely dense" categories for study eligibility. Eligible subjects had a clinical breast exam and mammogram with BIRADS classification of Negative, Benign, or Indeterminate but Probably Benign. High risk was defined as ≥ 3.5% risk of breast cancer over the next 5 years, as per the Gail model or the BRCAPRO genetic risk model. The average age at enrollment was 41.4 years and the average 5-year risk level was 10.4%. Twelve and 10 subjects, respectively, had breast MRIs with BIRADS category of 1 and 2, and underwent no further imaging. Zero subjects had MRIs with BIRADS category 4 or 5. Eight subjects had category 3 MRI results. Three of these underwent sonography which showed normal results and had no further workup. One subject had an ultrasound and cyst aspiration, with no evident pathology. One subject elected for 6 month follow-up. Three subjects had sonography with normal results, but underwent core biopsy (2) or fine-needle aspiration (1). One subject had invasive ductal carcinoma, not detected on other imaging. Two subjects had benign results.

Our preliminary experience indicates that 0.5T breast MRI can enhance surveillance for high-risk women with dense breasts. Our results are consistent with studies using higher strength magnets that show improved sensitivity, and suggest that this can be achieved without greatly sacrificing specificity.

205 Early Detection of Breast Cancer: Who is Responsible?

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Introduction:The early diagnosis and treatment of breast cancer is necessary to minimize the morbidity and mortality rates. To reach this goal we have two poles with the same responsibility: the patient and the medical team.

Objectives:1)To determine the general population knowledge about screening in breast cancer.2)To determine the diagnostic and therapeutic routes that non-oncologist physicians take with the patient with suspicious breast lesion.

Methods:We applied direct interviews to 250 apparently healthy women to evaluate their knowledge in breast cancer screening, and asked to 50 breast cancer patients about their prior medical proceedings.

Results:In the group of healthy women we found that 67% knew the meaning of mammography, but only 37% knew their indications, 73% knew the meaning of a breast mass and 41% of a nipple discharge, but only 36% made themselves a monthly breast examination. In the group of breast cancer patients we found 40% of them had at least high school level; 78% of the group were seen by 1 or 2 physicians before the right diagnosis; 65% expent more than 6 months to reach this diagnosis; 18% of the physicians were general practitioners and 39% gynecologists; 48% of the patients were seen for the first time at our institution in clinical stages III and IV.

Conclusions:With this study we found a knowledge problem in the general population and, unexpectedly, in the first contact physicians; this findings demonstrate a dangerous attitude of both groups to the problem increasing the number of patients with advanced disease.

206 Cost Analysis of Stereotactic Vacuum Assisted Core Biopsy (SVAC) Vs Stereotactic Large Core Excisional Biopsy (Site Select) Vs Needle Localization Biopsy (NL) by Pathologic Outcome.

Hughes KS, Smallman J, Germaine T, DeAngelis EA, Dedrick CG, Rolfs AT, Sites VR, Moskos MM, Sabo S, Robinson C.

When mammographic lesions require histologic evaluation, the goal is to obtain a definitive diagnosis with the least cost and the fewest procedures. The purpose of this paper is to determine the cost, and number of procedures for SVAC, NL or Site Select based on the ultimate pathologic outcome.

Methods: Patients with BIRADS IV and V mammograms treated by a single surgeon (KSH) between April 1, 1999 and April 30, 2000 were reviewed. Prior to December 13, patients were selected to have either SVAC or NL and after December 13, Site-Select became a 3rd option. Average billing at our facility was found to be: SVAC $3,017, Site Select $2,924, NL $4,341, Partial Mastectomy $4,508, and Partial Mastectomy plus NL $5,453. Total billing per patient (excluding axillary surgery) was determined by adding the bills of the initial and all subsequent procedures performed. Atypical hyperplasia (AH) could be lobular or ductal, or lobular carcinoma *in situ*.

Results:

	Site Select	SVAC	NL
Benign without atypia			
n	19	36	28
Average cost	$2,924	$3,379	$4,341
Average # procedures	1	1.1	1
Atypical Hyperplasia			
n	1	8	9
Average cost	$2,924	$7,360	$4,341
Average # procedures	1	2	1
Malignant			
n	7	10	24
Average cost	$6,596	$9,155	$6,032
Average # procedures	1.7	2.3	1.4

Conclusions: For AH and malignant disease, the most cost-effective approach requiring the fewest invasive procedures appears to be either Site-Select or NL. For benign disease, SVAC appears to be cost-equivalent to Site-Select, with more procedures per diagnosis; both are less expensive than NL. The choice of biopsy technique should be individualized, based on the level of mammographic suspicion.

207 An Evaluation of Complications of Stereotactic Vacuum Biopsy with the Mammotome of the Breast.

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Purpose:

Are there risk factors for complications in the use of the stereotactic vacuum biopsy with the Mammotome?

Material and Method:

Between January 7th, 1998 and March 10th, 2000, we performed 306 stereotactic vacuum biopsies with the Mammotome Vacuum Biopsy System. Our aim was to remove at least 80% or more of the suspect lesions such as microcalcifications and masses. All of the 306 procedures were retrospectively analyzed.

Results:

Altogether 10 punctures (3.0%) had to be broken off ahead of time. From these, 7 punctures were broken off because of severe bleeding, one patient needed a surgical intervention in the OR. In one case we had to stop because of an acute anxiety attack. 2 patients required dermal suture under local anesthesia due to skin aspiration. During 2 procedures we registered technical difficulties with the vacuum control module. In 10 cases we had to use 2 needles due to a blunt rotation knife inside the needle.

Conclusions:

The stereotactic vacuum biopsy with the Mammotome is an outstanding suitable method for the histological clarification of mammographically unclear or suspect findings of the breast. Risk factors for complications are small breast size, lesions close to the skin or thoracic wall and axillary or retroareolar lesions.

208 Surgical Assessment of the Surrounding Breast and Axilla after a Tumor-Positive ABBI Biopsy.

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Between January 1998 and April 2000 we performed 183 ABBI biopsies (13 using the 1.5 cm cannula, the rest by means of the 2 cm cannula). Of them, 48 (26.2%) revealed the presence of tumor (20 infiltrating ductal carcinomas, 1 lobular infiltrating carcinoma, 1 carcinoma of the pure tubular type, 23 ductal in situ, and 3 lobular in situ carcinomas. Thirty-three patients (18 with invasive cancer and 15 with in situ carcinoma) were reoperated by us.

Results:

a) Invasive carcinoma: Of 18 patients reoperated by us, 12 had ABBI biopsies with histologically free margins. This was confirmed when analyzing the surrounding breast tissue in 10 cases. All were submitted to a complete axillary dissection (number of obtained nodes 21-38). Eight patients were node-negative. The remaining one, hosting a 6 mm tumor, showed massive invasion in 9 out of 21 nodes, six of them with capsule rupture. Two patients with ABBI biopsies showing apparently free margins had tumor in the surrounding breast (1 in situ, 1 invasive carcinoma). Both were node-negative. Six patients had invaded margins in their ABBI biopsies. The surrounding breast tissue contained tumor in all instances (4 in situ, 2 invasive). Five axillary lymphadenectomies were carried out in this group: 2 had invaded nodes, and 3 were node-negative.

b) In situ carcinoma: 15 out of 26 patients were reoperated by us. Four showed tumor-free margins in the ABBI specimen. None had residual tumor. The other 11 patients carried apparently diffuse in situ tumors, reaching the biopsy margins. Residual tumor was diagnosed in 10 cases, one of them showing a focus of invasive tubular carcinoma. Six level-1 lymphadenectomies were carried out in this group, and all patients were node-negative.

Conclusions: Histologically invaded margins are highly suggestive of residual tumor after an ABBI biopsy. Histologically free margins, unfortunately, do not guarantee the absence of residual tumor. A very small invasive carcinoma, with ample margins and no residual tumor, furthermore, was associated with massive axillary invasion.

209 Cytologic Diagnosis of Nipple Discharge by Intragalactophoric Aspiration Method.

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The conventional cytologic diagnosis of nipple discharge samples has been most successful with accuracy around 60%, to increase the diagnostic accuracy from the patient with pathological nipple discharge using a simple intragalactophoric aspiration method.

We compared the cytodiagnostic accuracy, through the conventional method with squeezing collecting method and intragalactophoric aspiration by intravenous catheter (Hou MF et al, Radiology 1995, 195; 568-569), in a total 187 patients with spontaneous nipple discharge in a single duct without mass were pathologically identified at Kaohsiung Medical University Hospital, Taiwan.

Adequate specimens were collected in 97.5% (179/187 cases) of the sample by intragalactophoric aspiration method, compared to 75.9% collected by the conventional squeezing method ($p<0.05$). The cytologic diagnosis was divided into five categories; positive in 12 case, suspicious in 12, negative with atypical finding in 76, negative in 42 and inadequate specimens in 45 cases which employed the squeezing method. These results were less satisfactory findings than with the intragalactophoric aspiration method whose smears were positive in 22 cases, suspicious in 18, negative with atypical finding in 100, negative in 41 and inadequate specimen in 6 cases. Our results showed a sensitivity of 92% and specificity of 93% for intragalactophoric aspiration method, compared to a sensitivity of 52% and specificity of 89% for the squeezing method. Of the 38 cancers in our series, correct cytodiagnosis, including the suspicious cases, was made in 34 cases by intragalactophoric aspiration, with an accuracy of 89.5% compared to the 34.2% (13/38) accuracy rate of the conventional squeezing method ($p<0.05$).

For patients with pathological nipple discharge, the intragalactophoric aspiration method provides a much more accurate cytodiagnosis than does the conventional squeezing method.

210 Quantitative Immunohistochemistry with Antibodies to Phosphorylated Histone 3: A Novel Method to Identify Mitotic Cells in Tissue Sections.

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Determination of mitotic counts is a component of the Nottingham grading system for breast carcinoma and an important determinant of clinical outcome. However, several studies have demonstrated the variability of mitotic figure counts. Factors contributing to this variability include time interval prior to tissue fixation, fixation type and duration, and interobserver variability in assessment of mitotic figures. Thus, identification of a reliable M phase marker in tissue sections would be useful. Phosphorylation of the nuclear protein histone 3 (p-H3) and subsequent chromosomal condensation is an important step in mitosis that is highly associated with the M phase of the cell cycle. We wished to test the hypothesis that quantitative immunohistochemistry (IHC) using anti-p-H3 antibodies would be a potential replacement for mitotic figure counting. The anti-p-H3 antibody (Upstate Biotechnology) was applied to deparaffinized, formalin fixed tissue sections of normal skin and intestine, following heat induced epitope retrieval, to validate the technique. Subsequently, the antibody was applied to a series of 45 breast cancers that had also had mitotic indices determined as well as Ki-67 indices determined via IHC with the MIB-1 monoclonal antibody.

Scattered cells in the appropriate loci (basal layer of skin, base of crypts in intestine) were immunostained with the anti-p-H3 antibody. In the breast cancer specimens, it was noted that more cells were identified by anti-p-H3 immunostaining than by mitotic figure counting. Two distinct subpopulations of p-H3-positive cells were detected: one showing strong uniform nuclear signal, and the other showing a speckled nuclear pattern. Detection and counting of p-H3-positive cells was more easily performed than the counting of mitotic figures, with lower interobserver variability. While some correlations were found between p-H3 indices, mitotic figure counts, and Ki-67 indices, significant discordances were found in a subset of tumors.

These results suggest that traditional mitotic figure counts may undercount true mitotic activity, and that antibodies to p-H3 may represent a superior marker for the identification of mitotic cells in deparaffinized formalin fixed tissue. Additional studies comparing clinical outcome as a function of p-H3 indices versus mitotic figure counts or Ki-67 indices will be required to determine the ultimate value of p-H3 IHC.

211 Squamous Cell Cancer (SCC) - A Detailed Clinical, Pathologic and Molecular Assessment of 17 Cases.

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SCC of the breast is extremely rare and knowledge regarding its origin, clinico-pathological details, molecular markers (MM) and optimal treatment are limited. We have retrospectively identified and reviewed 17 cases of SCC diagnosed in Canada between 1981 and 1999. All cases were women with a mean age of 61 years (r 35-86); 6 were premenopausal and 11 were postmenopausal. In all cases, diagnosis was obtained through surgical resection and central pathological review including contemporary MM has been performed. Mean size of the primary identified in 16 patients was 3.3 cm (r 1.3-6.2); nodal status was: 10 - negative, 5 - positive and 2 - not determined; ER/PR status identified in 15 patients was negative in 13 (87%). Mean follow-up for all patients was 3.4 years. Treatment was as follows: surgery (94%), adjuvant chemo (47%) and adjuvant radiation (59%). Four patients have been lost to follow-up. Of the 13 remaining, 9 are alive and NED (mean DFS 3.4 y); 4 have died - 2 from metastatic SCC. MM including p-53, cerb-2, myb-1 and bcl-2 have been performed and these results will be correlated with conventional histopathological features such as grade, tumour size and SBR and will be reported. Similar to the conclusions of other authors, SCC is a rare but verifiable variant of breast cancer. This study, coupled with MM offers further insight into the clinico-pathologic behaviour of this entity.

212 The Implications of Malignant Alterations in Cytoskeletal Proteins on Keratin-Based Analyses in Breast Cancer.

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Cytokeratins (keratins, k) are the specific intermediate filament proteins exclusively expressed in epithelia. As markers for epithelial cells these cytoskeletal proteins are used as an important diagnostic tool for the analysis of bone marrow micrometastasis or in flow cytometry in breast cancer. During the malignant progression and dedifferentiation, however, a transition from epithelial to mesenchymal phenotype occurs. The loss of keratins might not only impair prognosis, but also affect the results of keratin-based analysis. Therefore we analyzed in 80 primary invasive ductal breast carcinomas as well as in 6 breast cancer cell lines immunohistochemically (APAAP) the expression of keratins K8, K18 and K19, characterizing luminal breast epithelia and vimentin, characterizing mesenchymal tissue. In relation to the protein expression disease-free (DFS) and overall survival (OS) over a ten-years-follow-up was evaluated for the tumors. For the cell lines the metastatic potency was assessed. In this context the influence of the oncoprotein HER2 on the keratin pattern was also analyzed.

A significant supression of the lumenal keratins was observed in the majority of the tumors (K8: 72,5%, K18: 83,7%, K19: 65%). Moreover 21,3% of the tumors aberrantly expressed vimentin. The loss of K8 and K18 as well as the expression of vimentin were associated with early metastasis and poor prognosis (OS: K8:p=0,004, K18:p=0,018, Vim:p=0,006). HER2 overexpression, seen in 35% of the cases, was significantly coexpressed with K19 (p=0,0004) and vimentin (p=0,0005). In the cell lines a similar change from keratin to vimentin expression was observed with increasing metastatic potency.

In breast carcinoma the increase in metastasis is associated with a dramatic decrease of epithelia-specific keratins and an upregulation of mesenchymal vimentin. These structural alterations are enhanced by the overexpression of HER2. This loss of keratins can potentially impair the results of keratin-based analyses of bone marrow micrometastasis, especially in HER2-overexpressing tumors with bad prognosis, as the most malignant, keratin-negative, vimentin-positive tumor cells might escape the detection.

213 Infrared Spectroscopic Imaging: A New Tool for the Pathology Laboratory.

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Infrared (IR) spectroscopy is growing in popularity as a technique for distinguishing tissue types and diagnosing disease states, including breast cancer. The development of IR array detectors has received substantial funding from the military over the past two decades. Until recently, much of this technology was classified. The combination of an IR focal-plane array detector and a Fourier transform infrared (FT-IR) microscope has recently proven to be a powerful one for obtaining spectroscopic images with unprecedented image fidelity. These images could complement assays such as HER-2/neu, VEGF, and hormonal receptor status.

We describe the use of inexpensive IR-reflective glass microscope slides that are still transparent in visible light. These slides allow us to perform FT-IR microspectroscopic imaging studies on samples which are also able to be evaluated by conventional pathology before or after collecting the IR images. Examples of micro-reflectance FT-IR spectroscopic images of H&E stained breast tissue biopsies will be presented.

214 Radial Scars: Correlation of Morphology, Imaging and Tumor Markers.

Kent SA, Wiley EL, DeLeon P, Adler YT. Northwestern University Medical School, Chicago, IL.

Radiographic findings in radial scar (RS) mimic those found in breast cancer and frequently necessitate breast biopsy. Morphologic assessment of RS lesions is required to evaluate epithelial proliferative and malignant components.

We reviewed radiographic images and histology of 35 core biopsies, 52 lumpectomies and 3 mastectomies from 66 patients in which RS were present. Proliferative changes were characterized for each RS. Immunohistochemical studies for ER, PR, Cerb-B2, p53 and smooth muscle actin (SMA) were performed in 48 specimens (with adequate tissue).

Radiologic images of 63 patients with RS showed architectural distortion in 38%, calcifications in 56% and mass in 14%. RS was the primary lesion in 59 patients (71%) as determined by radiological-histological comparison. Benign proliferative changes were present in all RS. Calcifications were found in epithelial hyperplasias (62), cysts (23), DCIS (5), and atypical duct hyperplasia (ADH) (1) components of RS. ADH was found in 12 RS (18%), lobular neoplasia (LN) in 10 (15%), DCIS in 5 (8%) and invasive carcinoma (IC) in 1. 3 patients had LN, 1 had ADH 3 had DCIS, and 3 had IC elsewhere in the same specimen but not within the RS. ER activity was increased 61% of 33 RS studied, most often in cystic epithelial hyperplasia (16); PR activity was increased in 67% of 30. p53 was over expressed in 7 cases (15%): over expression was also present in non-RS areas in 2 cases. Cerb-B2 showed membranous positive reaction in 7 cases (19%) that ranged from 1-10% of cells. SMA stains revealed reduced myoepithelial cells in areas of epithelial proliferation of RS in 24 cases (63%) and SMA was present in sclerotic stroma of 16 cases (42%).

RS present as architectural distortion and /or calcifications and were the primary lesion found in 71% of patients studied. RS can display proliferative components that are associated with an increased risk for carcinoma including ADH, LN and DCIS. Subtle changes in ER, PR are present in a majority of RS. Alteration in markers associated with malignancy (Cerb-2 & p53) are found within a minority of RS.

215 Measurement of Breast Pain: Significant Descriptors from a Modified Short McGill Pain Questionnaire.

Khan SA, Apkarian AV. Surgery; Neurosurgery, Upstate Medical University, Syracuse, NY.

Breast pain (mastalgia) is a common and understudied condition, which may have an interaction with breast cancer risk (Plu-Bureau 1992). Further research on mastalgia requires the identification of significant, valid pain descriptors in a concise series of questions which can be administered repeatedly to participants in future prospective studies.

The present study was designed to identify pain descriptors which most successfully predicted the total breast pain score in women with mastalgia, and to derive a short series of questions that can be used to concisely measure breast pain.

Women presenting to the Breast Care Center of University Hospital, Syracuse were presented with a short form of the McGill Pain Questionnaire which we have specifically modified for breast pain (bMPQ). The bMPQ included 11 sensory and 4 affective descriptors, a visual analog scale (VAS), a present pain index (PPI), questions on the duration, frequency and cyclicity of the pain, a sketch diagram of the breasts from which painful area was calculated, 4 quality of life (QOL) questions, and 2 questions regarding use of pain medication, and presence of other pains. Data from 286 women who had no history of breast cancer, and admitted to experiencing breast pain over the past three months were analysed using stepwise regression to identify the most parsimonious model to capture the total pain experience.

The subjects ranged in age from 16 to 81 years (mean 46.2). Of these, 134 women described cyclic pain, whereas 152 said their pain was non-cyclic. Total breast pain (Qt) was calculated as the weighted sum of the components described above. There were no significant differences in pertinent pain descriptors in the cyclic and non-cyclic groups, although some sensory components (aching, heavy and tender) were higher for the cyclic group. The most parsimonious model for Qt was one including the %VAS, %PPI, and %QOL. The adjusted R^2 for this model was 0.96, and the p value for each of the three parameters was <.0000001.

Conclusions: We have used a standard pain measurement instrument (the short MPQ) to develop a succint series of questions which reliably capture pain data for use in epidemiologic studies of breast pain. Such studies are urgently needed to 1) clarify the significance of breast pain relative to breast cancer risk, and 2) investigate the etiology and treatment of this common and distressing condition.

216 Emotional Disturbance at the Time of Breast Biopsy: Is This a Teachable Moment?

O'Neill SM,[1] Davison D,[1] Rubinstein WS,[2] Vogel VG.[1] [1]Comprehensive Breast Program, University of Pittsburgh Cancer Institute and Magee-Womens Hospital (UPCI/MWH), Pittsburgh, PA; [2]Cancer Genetics Program.

Breast biopsy is an anxiety-producing event for most women. However, studies have shown that moderate anxiety can be a motivating factor in assuming proactive health behaviors, such as screening and surveillance, and that a major self-reported need of women undergoing breast biopsy is information and education about their future risk. This study followed 100 women attending a comprehensive breast program for fine needle, core, and stereotactic breast biopsies. After the biopsy procedure,subjects completed questionnaires measuring breast cancer knowledge and attitudes, and two psychological assessments;the Profile of Mood States(POMS) and the Impact of Events Scale(IES). Breast cancer risk assessment was also done using the Gail and Claus models.

Subjects had a mean age of 48,and were predominantly Caucasian (95%), college graduates (50%), and married (71%), with 75% reporting a very strong support system. A first or second degree relative with breast cancer was reported by 37%. The mean total mood disturbance score (TMD)on the POMS was 16, a score higher than the normative scores reported for psychiatric outpatients. In contrast, a group of 60 women at the same facility who completed the POMS after routine mammography procedures had a mean TMD <1. Highest scores were reported for the individual measures of tension/anxiety(8), fatigue(5.1), and confusion(4.8). Significant increases (ranging from 70% to 400%)were found in the scores for confusion, depression, tension/anxiety, and anger when the post-biopsy group was compared to the post-mammography group($p<0.0005$). The mean scores on the IES for intrusive thoughts and cognitive avoidance relating to the breast biopsy were 11, and 10.9 respectively; scores approaching those of diagnosed cancer patients.

These findings suggest that the time of biopsy may not be optimal for presenting complex educational material about breast health. Simple referral algorithms outlining next-step scenarios, including the option of risk counseling, may be sufficient to reduce anxiety without overwhelming patients at this time. Follow-up POMS and IES completed at 3-6 months post-biopsy resulted in a decrease of disturbance for most women. At this juncture, age appropriate breast health surveillance should be reinforced in order to allay fear related to future detection practices, and educational initiatives may be more productive.

217 Breast Cancer on the World Wide Web: Implications for Breast Cancer Specialists.

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Women diagnosed with breast cancer seek information from many sources. These sources of information traditionally include conversations with health professionals, leaflets and videos. The Internet has brought about a revolution in the way patients access information and there are several breast cancer support groups colonising cyberspace. Patients can find help on the Internet through entire breast cancer communities, who communicate by way of e-mails, newsgroups, bulletin boards and chatrooms. In addition, there are several web sites with information about breast cancer. These web sites are powerful sources of education and information. Unfortunately, the data is unregulated and carries the risk of being inaccurate. Hence, in some cases these web sites by providing misleading information may lead to adverse care [1,2] In addition, there are concerns that this misleading information may create patient doubt and distrust [1] .

In order to reduce this potential for misinformation and therefore increased patient anxiety, we developed a website, designed and created by health care professionals. The site is aimed specifically at patients attending the breast clinic at St Margarets Hospital, but can also be used as a comprehensive source of information for any patient with breast problems world-wide.A questionnaire was developed to assess the response to the website among our patients and sent out to 200 patients. Results will be presented.

In view of our experience with our web site providing information on breast cancer for patients, we believe the internet is going to play an ever increasing role as a source of information. Hence, we recommend that breast cancer specialists be aware of this and should either identify [3] or create web sites with accurate information for the benefit of their patients.

References:

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218 Impact of Consumers on Scientific Review of Breast Cancer Research Proposals.

Andejeski Y, Bisceglio IC, Dickersin K, Johnson JE, Robinson SI, Smith HS, Visco FM, Rich I, and the USAMRMC Fiscal Year 1995 Breast Cancer Research Program Integration Panel . Department of Defense Congressionally Directed Medical Research Programs, Fort Detrick, MD.

We evaluated the impact of having breast cancer survivors with advocacy experience (consumers) participate on the panels convened for scientific review of 2,206 proposals submitted to the Department of Defense (DoD) Breast Cancer Research Program in 1995. The process involved 561 scientists, 47 panel chairs, and 85 consumers, who reviewed the breadth of science from basic to clinical and behavioral disciplines. Each review panel had one chairperson, two consumers, and, generally, from 12 to 17 scientists. To assess the effects of consumer voting on proposal scoring, we compared scores assigned by consumers to those assigned by scientists + panel chairs. Mean consumer scores were more favorable than mean scientist + chair scores for 62.3% of the proposals, and less favorable for 35.4% of the proposals. Overall proposal scores, however, were more favorable than those that would have been obtained without consumer votes for 15.2% of the proposals, and less favorable for 8.6% of the proposals. For all but 13 of these proposals, the difference was ±0.1. (Scores were on a scale ranging from 1.0 to 5.0.)

We surveyed all participants before and after the panel meetings to assess opinions on how consumers affected the review process. Although pre-panel opinions tended to be positive, 10 of 15 comparisons showed that significantly greater proportions (all p values ≤0.01) of participants had positive post-panel opinions. Having consumers on the review panels was perceived to be beneficial (82.9%, 95.8%, and 96.5%, for scientists, panel chairs, and consumers, respectively) and to not have drawbacks (73.5%, 90.5%, and 87.3%, respectively). Our results support continued participation of consumers in the review of research proposals.

219 Some Reflections on Clinical Trials.

Hetrick VR. You Are Not Alone, Los Angeles, CA.

The purpose and effect of clinical trials are not well-understood in the patient community and especially are not well-understood outside of the patient community. Physicians and advocates need to set an objective of informing both patients and the public about the issues raised below and educating the media on the precise meaning of the results of clinical trials, whether the results are final or intermediate.

Only about three percent of adults participate in any type of clinical trial while well-over 50 percent of children with life-threatening diseases such as cancer participate in clinical trials. This difference has clearly led to much more rapid clinical implementation in non-trial settings for pediatric patients than for adults. Ideas about why this difference exists and ideas on how to increase the proportion of adults who participate in clinical trials are the objectives of this paper.

The reasons most frequently given for not participating in clinical trials are:

* My doctor never mentioned it to me;
* I do not want to be a guinea pig;
* I want the best treatment I can get for my disease; and
* The media say the clinical trial for my disease is not effective.

Not mentioned are:

* I do not understand what the term "clinical trial" means; and
* I do not understand what clinical trials means in the context of what is threatening my life.

While interviewing patient respondents to a recent survey questionnaire, we found that these patients believed educating their physicians and the media would be the two most useful tasks that advocates could set for themselves.

As a consequence, this presentation contains a proposal for accomplishing those educational tasks.

220 The Grassroots Fight to End Breast Cancer: Advocacy and Action.

Warner MD.

The National Breast Cancer Coalition (NBCC) is a grassroots organization committed to eradicating breast cancer through action and advocacy. Created in 1991, NBCC has grown to a nationwide network of more than 500 organizations and 60,000 individuals.

Through its goals to increase research, improve access to quality treatment and care and expand influence of trained breast cancer advocates, NBCC continues to strive toward ending breast cancer.

NBCC's advocacy efforts have increased federal funding for quality, peer-reviewed breast cancer research more than six fold in seven years – from $87 million before the Coalition began to more than $600 million in 1999. NBCC trains breast cancer activists to take leadership roles within the world of science and research. NBCC also develops programs to redesign the research process by focusing innovative breast cancer research on prevention, cause and cure.

NBCC's grassroots campaigns have brought about an unprecedented multi-million dollar breast cancer research program within the Department of Defense and a commitment from President Clinton to a National Action Plan on Breast Cancer – a public/private partnership designed by activists, scientists and policy makers.

NBCC's educational programs prepare members to participate in how breast cancer research funds are spent. Project LEAD, NBCC's innovative science training course for breast cancer advocates, provides activists with the skills needed to serve on influential research boards and committees in government, universities, hospitals and private industry. Through its Clinical Trials Project, NBCC ensures that breast cancer advocates are involved in the design and implementation of clinical trials. NBCC also works with industry and government to design, oversee and facilitate clinical trials.

In the hopes that there may be an increase in innovation and speed within the current research environment, NBCC hosts an annual Think Tank meeting at the Aspen Institute, bringing together a diverse group of visionaries to rethink approaches to breast cancer. From these meetings NBCC has made a commitment to quality health care for all people and has determined the core values of quality health care: Access, Information, Choice, Respect, Accountability, and Improvement. In addition, grassroots activists have joined NBCC to insist that the correct questions are being asked in the exploration of potential links between breast cancer and the environment.

221 BRCA1 and BRCA2 Mutations in Breast Cancer Patients of African American Descent.

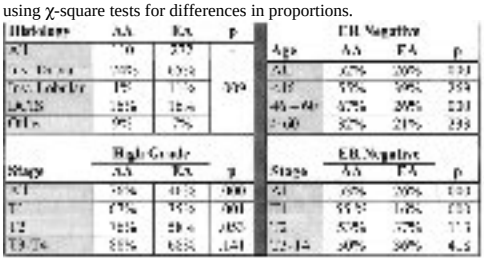
Haffty BG, Alvarez-Franco M, Silber A, Matloff E, Bale AE.

The frequency and significance of mutations in the BRCA1 and BRCA2 gene in women of African American descent has not been extensively evaluated. As part of an ongoing study, we have been actively recruiting patients with early onset breast cancer previously treated with conservative surgery and radiation therapy,unselected for family history, ethnicity, or race, to determine the frequency and clinical significance of germline mutations in BRCA1 and BRCA2. To date we have recruited 130 women and have results on over 100 women in whom sequencing of the entire BRCA1 and BRCA2 gene has been completed. Of the 130 particpants, 15 women are of African American descent, and results are complete on 9 of these African American women and 90 white women. All 9 African American women had early stage breast cancer with a median age of 40 years and a median follow-up of 7 years from date of initial diagnosis. Of the 9 African American women with sequencing results completed, seven have been found to have a mutation in either BRCA1 or BRCA2. Of interest, only one variant was found in BRCA1, while six were in BRCA2. Three of the BRCA2 variants were classified as disease associated mutations, and three are genetic variants of unclear significance. Two of these variants had previously been reported in African-American populations. The frequency of disease associated mutations and genetic variants of unclear significance, even in this population of young women with breast cancer, is higher than anticipated. Although the number of African American patients recruited to date in this study is small, the high frequency of BRCA2 mutations is notable and significantly higher than the mutation frequency in our population of white women ($p < 0.01$). If confirmed in a larger sample size, these results may have clinical and epidemiological significance. We are in the process of conducting a larger study, with 60 -70 additional African American patients with early onset/early stage breast cancer, to determine the clinical significance and frequency of BRCA1/2 mutations in this population.

222 Ethnic Differences in Estrogen Receptor (ER) Negativity, Tumor Stage, Grade and Histology in a Retrospective Breast Cancer Review.

Butler WM, Cunningham JE, Reynolds JA, Sweatman CA, for the South Carolina Comprehensive Breast Center.

In a retrospective review of the 384 new female breast cancer cases seen at our facility in 1998, we found substantial differences in tumor characteristics according to ethnicity. A total of 319 (83%) were invasive. Ethnic differences were assessed using χ-square tests for differences in proportions.



Proportionately, twice as many invasive tumors in African-American (AA) women were ER negative, compared to European-American (EA) patients. This pattern was observed in all age groups and tumor stages, statistically significant in the earliest (T1) breast cancers and perimenopausal years. AA women also had more high grade tumors, especially in T1 cancers. Invasive lobular carcinoma was seen less frequently in AA patients. With age, stage, ER and histology included in a regression model, race remained an independent predictor of high grade invasive tumors. We believe that a different biology may be present in early stage invasive breast cancer in AA patients and that this may account for some of the reported differences in survival for AA women.

223 Comparison of Breast Cancer Cases in the Arab-American and Caucasian Populations of Metropolitan Detroit.

Do HT, Kau T-Y, Weiss LK, Severson RK, Schwartz KL. Epidemiology, Karmanos Cancer Institute, Detroit, MI.

The Detroit metropolitan area has one of the largest populations of Arab-Americans in the United States, with estimates ranging from 250,000 to 400,000. Since this group is not routinely identified in US census data or in hospital records, only limited information on cancer incidence in this population is available. The goal of this project was to identify Arab/Chaldean cancer cases in the Detroit Surveillance, Epidemiology and End Results (SEER) registry, using a surname file similar to the Spanish surname file currently used by the National Cancer Institute (NCI), to determine proportional cancer incidence rates in this population. Arab and Chaldean surnames and first names were identified from sources such as telephone and membership directories, Michigan vital statistics, and the SEER database. Several Arab and Chaldean community outreach organizations assisted with this project.

Data on 47,309 cases of invasive female breast cancer diagnosed between 1975 and 1998 were selected from the Detroit SEER registry. Two hundred and thirty-five of these cases matched on both first and last name or maiden name and were identified as Arab-American. Breast cancer comprised a larger proportion of cancers among patients younger than 50 years of age in the Arab-Americans compared to Caucasians (40.3% vs 22.7%, p=0.001). A greater percentage of the Arab-American women were married (71.9% vs 56.1%, p=0.001). Significant differences in size and staging of the tumor at time of diagnosis were not present.

Based on a Cox proportional hazards analysis, stratified by age group (0 to 49, 50 to 64, 65+) and adjusting for marital status, tumor size and stage, Arab-Americans have better survival than Caucasians in all age groups (RR=0.61, CI 0.407-1.004; RR=0.68, CI 0.443-1.006; RR=0.60, CI 0.347-0.926, respectively).

This initial analysis indicates differences exist in breast cancer patterns among Caucasians and Arab-Americans. Additional studies must be conducted to evaluate potential reasons for these differences and assist in the development of appropriate intervention strategies.

224 Rate of Chemotherapy Related Amenorrhea (CRA) Associated with Adjuvant Adriamycin and Cytoxan (AC) and Adriamycin and Cytoxan Followed by Taxol (AC+T) in Early Stage Breast Cancer.

Stone ER, Slack RS, Novielli A, Ellis M, Baidas S, Gelmann E, Cohen P, Warren R, Stearns V, Hayes DF, Isaacs C. Georgetown University Hospital, Washington, DC.

To date, various studies have evaluated age related CRA. Most have focused on methotrexate-containing regimens. Only a few small studies have been published investigating the incidence of CRA with AC. No studies have been published looking at a taxane-containing regimen. We performed a retrospective analysis of 98 premenopausal women, age 50 or younger, treated with adjuvant AC or AC+T between 1992 and 1999. Premenopausal was defined as the presence of cyclic menstrual bleeding. CRA was defined as the absence of menses for one year after the initiation of chemotherapy. Patients with a history of prior chemotherapy, adjuvant chemotherapy followed by high dose chemotherapy or hysterectomy were excluded. The majority of patients were identified by cross referencing diagnostic codes and chemotherapy pharmacy records. Patients completed a questionnaire, which inquired about chemotherapy regimens, and menstrual history before, during and after chemotherapy. 65 women completed the questionnaire and information about 16 additional patients was obtained from their medical record (81/98; 83%). 60 women received AC(adriamycin 60mg/m^2 and cytoxan 600mg/m^2 for 4 cycles) and 21 received AC+T(AC followed by taxol 175mg/m^2 for 4 cycles). Mean age at the time chemotherapy was initiated was 41 (range 24-50). The overall incidence of CRA with AC was 43%(95% confidence interval [CI] is 30% to 56%). Age related CRA for AC was as follows: for <35-0/11 (0%); 35 to <40-2/14 (14%); 40 to <45-7/18 (39%); and 45-50-17/17 (100%). The overall incidence of CRA with AC+T was 38%(95% CI is 15% to 61%). Incidence for women <40 was 4/12(33%;CI is 2% to 65%) and ≥40 4/9(44%;CI 4% to 85%). We conclude that the incidence of CRA following AC increases with increasing age with a large proportion of women under the age of 40 retaining ovarian function. The addition of taxol does not appear to substantially increase the overall risk of CRA but larger studies are needed to make any definitive conclusions. The high rate of preserved ovarian function seen in young women following chemotherapy for early stage breast cancer with AC and AC+T is promising for those wishing to preserve fertility and prevent the complications of premature menopause. These results may also have greater implications in light of emerging information on the role of ovarian ablation in the adjuvant setting.

225 WITHDRAWN

226 Toxicity and Early Survival Results of a Prospective, Randomized Adjuvant Trial Comparing Toremifene and Tamoxifen in Node-Positive Breast Cancer.

Holli K,[1] Valavaara R,[2] Blanco G,[3] Kataja V,[4] Hietanen P,[5] Flander M,[6] Pukkala E,[7] Joensuu H.[5] [1]Department of Palliative Medicine, Tampere University Hospital, Tampere, Finland; [2]Department of Oncology, Turku University Hospital, Turku, Finland; [3]Department of Oncology, Oulu University Hospital, Oulu, Finland; [4]Department of Oncology, Kuopio University Hospital, Kuopio, Finland; [5]Department of Oncology, Helsinki University Hospital, Helsinki, Finland; [6]Department of Oncology, South Karelia Central Hospital, Lappeenranta, Finland; [7]Finnish Cancer Registry, Helsinki, Finland.

The Finnish Breast Cancer Group initiated an adjuvant trial comparing two antiestrogens in postmenopausal patients with axillary node positive breast cancer in 1992. In this multicenter trial, toremifene at a dose of 40mg/d, is compared to tamoxifen at the standard dose of 20mg/d for three years. The first 899 patients (of 1480 total), with mean follow-up time of 3.4 years were included the scheduled safety and efficacy analysis. The enrollment for the trial was completed in June 1999. There was a trend toward more vascular complications (deep vein thromboses, cerebro-vascular events, and pulmonary embolisms) in tamoxifen treated patients (5.9%) than in patients treated with toremifene (3.5%), (p=.11). Bone fractures, on the other hand, tended to be more common in the toremifene group. The number of subsequent second cancers was similar. The subjective side-effect profile was similar in both treatment groups, sweating and hot flashes were most common during the first 6 months of therapy. Weight gain increased during the first two years in both groups but was decreasing about the entry level after that. The breast cancer recurrence rate was 23.1% (n=106) in the toremifene group and 26.1% (n=115) in the tamoxifen group (p=.31). The mean time to breast cancer recurrence and overall survival were similar for both groups of patients. This planned toxicity analysis suggests that toremifene is effective and safe for the adjuvant treatment of node-positive breast cancer in postmenopausal women. The small differences seen in efficacy and side-effects may be explaned by the different estrogen-antiestrogen ratio between toremifene and tamoxifen.

227 Cognitive Changes and Menopausal Symptoms of Women Receiving Adjuvant Chemotherapy for Breast Cancer. Early Results of a Matched Cohort Study.

Tchen N, Downie FP, Theriault M, Tannock IF. Medical Oncology and Hematology, Princess Margaret Hospital, Toronto, ON, Canada.

Most women with breast cancer receive adjuvant chemotherapy which has been shown to improve survival, but induces several types of toxicity. Improvements in supportive care have decreased commonly-reported toxicities, but there is growing evidence for the importance of more subtle effects such as chronic fatigue, cognitive changes (referred to by patients (pts) as 'chemo-fog' or 'chemo-brain') and symptoms associated with an accelerated menopause. Our preliminary study (Brezden et al, JCO, in press July 2000) and that of Schagen et al (Cancer 1999;85:640-650) have provided initial evidence for substantial cognitive changes in some pts.

The objectives of the present study are to determine the incidence and severity of (a) cognitive changes and (b) menopausal symptoms in women receiving adjuvant chemotherapy. 100 women who are receiving adjuvant chemotherapy are being recruited to this matched cohort study. Each pt recruits a relative, friend or neighbour, matched by age, as a control. Pts and controls complete the following assessments under the guidance of a research assistant: the High-Sensitivity Cognitive Screen (HSCS), the Functional Assessment of Cancer Therapy- general quality of life scale (FACT-G), with the subscales for endocrine function (FACT-ES) and fatigue (FACT-F). They also perform a computer-based test to evaluate reaction time. Since January 2000 we have approached 78 pts and recruited 51 pts able to identify a control. Four pts refused, 11 were not sufficiently fluent in English and 12 were unable to identify a control. We will present the first results of the study with demographic data, the distribution among pts and controls of the six individual cognitive domains of the HSCS (memory, language, visual-motor, spatial, attention and concentration, and self-regulation and planning), and the global score measuring overall cognition. We will also present a multivariable analysis of the influence on the summary HSCS score of potentially important factors (age, education level, emotional well-being, fatigue, menopausal status and symptoms and type of chemotherapy).

This study generates information that will be used to provide information and counseling for patients who will receive adjuvant chemotherapy.

228 The European Table-Study-Group: Safety Data and Serum Hormone Levels of the Adjuvant Therapy in Premenopausal Breast Cancer Patients Comparing Leuprorelinacetate and CMF.

Untch MM,[1] Wallwiener DD,[2] Schmid PP,[3] Sobotta KK,[1] Bondar GG,[4] Kienle EE,[5] Possinger KK,[3]. [1]Obstetrics and Gynecology, Ludwig-Maximilian-University, Munich, Germany; [2]Medical Clinic II, Humboldt-University, Berlin, Germany; [3]Oncology, Antitumor Centre of Donezk, Donezk, Ukraine; [4]Takeda Pharma, Aachen, Germany; [5]Ostetrics and Gynecology, University, Tuebingen, Germany.

The current adjuvant standard therapy for node-positive, premenopausal breast cancer patients is based on chemotherapy. The EBCTCG metaanalysis showed that ovarian ablation also results in a significant prolongation of survival. A reversible medical form of ovarian ablation would be a more acceptable treatment option than surgical oophorectomy. The TABLE-Study was an open, randomized multicentre phase III trial for node-positive(N1-9), hormone-receptor-positive, pre- or perimenopausal patients. A two year treatment with the GnRHa Leuprorelinacetate 3-month-depot (LAD) is compared with CMF-polychemotherapy. 600 patients have been enrolled. For patients under leuprorelinacetate the most dominating symptoms of oestrogen suppression were as expected hot flushes (80% vs. 47%), nervousness (38% vs. 27%), weight increase (80% vs. 47%) and increased sweating(77% vs. 52%). In contrast, patients with CMF complained mainly of nausea (86% vs. 16%), vomiting (56% vs. 6%), alopecia (62% vs. 16%) and loss of appetite (53% vs. 7%). LH levels were significantly lower under LAD compared to CMF during the entire treatment period. The suppression of estradiol starting 3 months after beginning of therapy and followed up to over 24 months was 25-30% more effective under leuprorelinacetate than under CMF treatment (postmenopausal estradiol after 6 months: 90% vs. 62%; after 12 months: 90% vs. 65%; after 24 months: 97% vs. 74%). The CMF-group showed lower pogression rates in patients with complete suppression of estradiol levels (6% vs. 28%). The ovarian ablation with GnRH analogues offers a new treatment option to premenopausal breast cancer patients compared to CMF (Jakesz, ASCO 1999) and compared to anthracycline containing regimens (Roche, ASCO 2000). Durable hormonal effects translate into significant response rates, compared to the only transient endocrine effect of chemotherapy. Data on relaps free and overall survival will underline this approach.

229 Do Women with 4 or More Involved Axillary Nodes Need Taxol or Just More Adjuvant Chemotherapy?

Cameron DA. Oncology, Edinburgh University, Edinburgh, Scotland, United Kingdom.

Since the results of the US intergroup-0148 study were first presented at ASCO in 1998, Adriamycin/Cyclophosphamide followed by Taxol (AC-T) is considered by many to be standard adjuvant therapy for node-positive breast cancer. However, sub-group analysis demonstrates a DFS and OS benefit for the addition of taxol only in the ER-negative patients not receiving tamoxifen. One hypothesis is that this added benefit relates to the longer duration of chemotherapy and not specifically from the taxol. Furthermore, NSABP B-15 confirmed that 4 cycles of AC gives comparable outcome to 6 cycles of classical CMF, a regimen found to be inferior to other anthracycline-containing regimens of similar duration. Until the completion of trials comparing AC-T with a non-taxane regime of identical duration, the true added benefit of the taxol remains uncertain. An indirect answer concerning drugs and schedule can be obtained by comparing the outcome data from other randomised anthracycline-based adjuvant trials. The most comparable data comes from the Milan study which found a significant improvement in DFS & OS for the sequential arm 4*A-8*CMF as compared with alternating Adriamycin & CMF in women with 4 or more involved axillary nodes. The patients in this Italian study were broadly similar to those in the 0148 study, with 1/3 post-menopausal and around 70% receptor positive (37% and 66% for the Intergroup 0148 study). Therefore the outcomes of women with 4 or more involved nodes have been compared for the two studies (using data for the Intergroup study in BMS literature, and the published graphs for the Milan study).This analysis suggests that although the AC-T regimen is a clear improvement over 4 cycles of AC alone, the sequence of 4*A-8*CMF gives comparable results. Thus although taxol improves the outcome for at least some patients as compared to 12 weeks of AC, similar benefit might be anticipated from the use of the much cheaper and well-tolerated CMF regimen. 1. Henderson IC,et al. Proc ASCO 1998; 17:101 (abstract 390A 2. Fisher B, et al. J Clin Oncol 1990; 8:1483-96 3. Levine MN, et al. J Clin Oncol 1998; 16:2651-8 4. Bonadonna G, et al. JAMA 1995; 273:542-7 5. Bristol-Myers Squibb Oncology. Jan 2000.Princeton, New Jersey

2-year	AC	AC-T	4xA-8xCMF	Alt. a/CMF
OS	89%	92%	93%	88%
4-9 node DFS	80%	85%	86%	76%
10+ node DFS	64%	69%	69%	66%

230 High Dose Epirubicin and Cyclophosphamide (EC) Vs Cyclophosphamide, Methotrexate, Fluorouracil (CMF) in High Risk Premenopausal Breast Cancer Patients: 5-Year Results of a Prospective Randomized Trial.

Galligioni E, Cetto G, Crivellari D, Nascimben O, Buonadonna A, Molino A, Lucenti A, Graiff C, Barni S, Puccetti C, Ferrazzi E, Recaldin E, Sava C, Saracchini S, Sacco C, Talamini R, and the GOCNE (Gruppo Oncologico Cooperativo del Nord Est). Italy.

Young premenopausal patients with 4 or more involved lymphnodes represent a particular group of high risk patients (pts). In the attempt of improving the modest effect of adjuvant chemotherapy in these pts, we randomized,between 1/90 and 4/95 207 consecutive premenopausal breast cancer pts pT1-3, with >3 involved lymphnodes, to classic CMF for 6 cycles or to EC (E 120 mg/sqm and C 600 mg/sqm day1 q 21) for 4 cycles. No hormonal therapy was planned at the end of chemotherapy. Median age, tumor size, number of involved lymphnodes (≤10, >10), estrogen receptor status and type of surgery were well balanced among the 104 CMF and the 103 EC pts . Data on toxicity, received dose intensity and 3 yrs results have been already reported (ASCO 1997). After a median follow-up in excess of 5 years, 13 % local relapses were observed in EC and 16% in CMF pts. Distant metastases developed in 46 % of EC and in 52% of CMF pts . Median DFS was 4.2 yrs for CMF and 5.5 yrs for EC pts (6.3 and 8.7y for N≤10, 2.3 and 3.4y for N>10 respectively). These differences are not statistically significant. Median Overall Survival was 6.8 yrs for CMF and 8.3 for EC pts (5.7 and 6.8y respectively for N>10). At multivariate analysis (Cox model) EC was superior to CMF in terms of survival (RR= 1.45, p= 0.0238). Data on cerbB-2 expression were reviewed in most pts and the analyses will be available by the time of the meeting. In conclusion, 4 cycles of EC, at the doses used in this study, seems more effective than 6 CMF cycles and apparently able to improve survival in premenopausal high risk breast cancer pts.

231 Tumor Biological Factors uPA and PAI-1 as Stratification Criteria for Risk-Adapted Adjuvant Chemotherapy: Second Interim Analysis of a Randomized Multicenter Trial in Node-Negative Breast Cancer.

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Tumor invasion factors uPA (urokinase-type plasminogen activator) and PAI-1 facilitate metastasis and are associated with a strong prognostic impact in node-negative breast cancer. A prospective randomized multicenter therapy trial in node-negative breast cancer (Chemo N0) was started in Germany in June 1993. Recruitment continued until the end of January 1999. Patients with low uPA and PAI-1 did not receive any adjuvant systemic therapy. Node-negative breast cancer patients with high levels of uPA and/or PAI-1 in the tumor tissue were randomized to adjuvant CMF chemotherapy versus observation only. The first interim analysis, performed in 556 patients after a median follow-up of 32 months, confirmed significant prognostic impact of uPA and PAI-1 in node-negative breast cancer. Patients with low uPA and PAI-1 levels had an estimated 3-year recurrence rate of 6.7%, whereas the rate was more than twice as high (14.7%) in patients with high uPA and/or PAI-1 (p=0.006). The Kaplan-Meier survival curve for DFS is remarkably similar to that obtained after a long-term follow-up in a unicenter trial in node-negative breast cancer patients without adjuvant systemic therapy. In the randomized patients (n=182), a substantial reduction in the estimated probability of disease recurrence at 3-years attributable to adjuvant CMF was observed. In the intention-to-treat analysis, the treatment benefit attributable to adjuvant CMF lacked statistical significance whereas the benefit was already statistically significant in the per-protocol analysis (p=0.016; RR=0.27, 95% CI: 0.09 - 0.78). It is expected that the treatment effect will become statistically significant with the longer median follow-up, available at the upcoming analyses.

The second interim analysis, 6 years after start of the trail is now being performed. It covers 689 patients, of whom 249 were randomized We anticipate that the results of the Chemo N0 trial will have a profound impact on clinical practice regarding prognosis assessment and risk-adapted individualized treatment strategies in node-negative breast cancer.

232 The ATAC ('Arimidex', Tamoxifen, Alone or in Combination) Trial: The Effectiveness of Transvaginal Ultrasonography and Diagnostic Hysteroscopy in the Prediction of Endometrial Abnormalities in Asymptomatic Postmenopausal Women.

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Introduction: The ATAC Trial is a randomised, double-blind trial comparing 'Arimidex' (anastrozole), alone or in combination with tamoxifen, relative to tamoxifen alone as 5-year adjuvant treatment for postmenopausal women with early breast cancer. The ATAC Endometrial Sub-Protocol was initiated to establish the background incidence of pathology and assess prospectively the incidence and nature of intra-uterine changes following endocrine therapy. There is no consensus of opinion as to the best way of screening the endometrium of asymptomatic women or those taking tamoxifen or other endocrine therapy. This study aims to assess the effectiveness of transvaginal ultrasonography (TVUS) and hysteroscopy in the prediction of endometrial abnormalities in asymptomatic women.

Methods: 285 gynaecologically asymptomatic women entered the sub-protocol. 262 of them had a TVUS and a hysteroscopy was attempted on 260. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) are reported for both techniques.

Results: 254 women had endometrial biopsies suitable for inclusion in the analysis. 21 women had histologically diagnosed endometrial abnormalities (20 polyps, 1 atypical hyperplasia). The sensitivity of TVUS for the diagnosis of endometrial pathology was 14.3% and the specificity was 97.8% (PPV 37.5%, NPV 92.5%). Hysteroscopy had a sensitivity of 90.5% and a specificity of 96.5% (PPV 70.4%, NPV 99.1%). The sensitivity and the specificity of TVUS for the prediction of hysteroscopic abnormality were 27.3% and 99.6% respectively (PPV 90%, NPV 90.2%).

Conclusion: This study provides data on the effectiveness of TVUS and hysteroscopy in gynaecologically asymptomatic postmenopausal women. In this group of women, hysteroscopy appears to be superior to TVUS for the diagnosis of endometrial pathology.

233 The ATAC ('Arimidex', Tamoxifen, Alone or in Combination) Trial: Transvaginal Ultrasound Scan Findings Overestimate Observed Pathological Findings in Postmenopausal Gynaecologically Asymptomatic Women before Treatment.

Jackson TL, Duffy SRG, on Behalf of the ATAC Trialists Group (Endometrial Sub-Protocol). Division of Obstetrics and Gynaecology, St James's University Hospital, Leeds, West Yorkshire, United Kingdom.

Introduction: The ATAC Trial is a randomised, double-blind trial comparing 'Arimidex' (anastrozole), alone or in combination with tamoxifen, relative to tamoxifen alone as 5-year adjuvant treatment for postmenopausal women with early breast cancer. The ATAC Endometrial Sub-Protocol was initiated to establish the background incidence of pathology and assess prospectively the incidence and nature of intra-uterine changes following endocrine therapy. Detection of abnormalities was investigated using transvaginal ultrasound scan (TVUS) and hysteroscopy. The baseline TVUS findings are presented.

Methods: 285 gynaecologically asymptomatic women entered the sub-protocol. 262 of them had a TVUS. Endometrial thickness and texture were recorded. The uterine dimensions were measured. Presence of fibroids, polyps and ovarian cysts were noted.

Results: Mean uterine volume overall was 48.1cm^3 ranging from 8.5cm^3 to 276.6cm^3. 83 women (32%) had fibroids with a mean uterine volume of 68.6cm^3. Those without fibroids had a mean volume of 37.4cm^3. 10 women were thought to have polyps with a mean volume of 49.2cm^3. Those who had taken HRT had a mean volume of 53.2cm^3 compared with 44.7cm^3 if not on HRT. Mean endometrial thickness was 4.8mm (range 1mm to 65mm). 32 women in total (12%) had heterogenous endometrium and 59 (23%) had endometrium thicker than 5mm. 21 women (8%) had endometrial pathology in the group (18 benign polyps, 1 polyp with atypia, 1 polyp with simple hyperplasia and 1 atypical hyperplasia). Of these, 7 had heterogenous endometrium and 9 had endometrial thickness greater than 5mm. One or both ovaries could not be visualised in 76 women (29%). 21 women had ovarian cysts, 2 of which were considered suspicious.

Conclusion: This is the largest group of asymptomatic postmenopausal patients with breast cancer to be described. TVUS is often suggested as a useful screening investigation in postmenopausal women. Factors such as endometrial thickness above 5mm and endometrial heterogeneity usually result in further investigations. In this group of gynaecologically asymptomatic women up to 23% may have been subjected to further investigations on the basis of their scan despite only 8% having pathology. The difficulty in visualising postmenopausal ovaries was confirmed.

234 The Incidence of Neutropenic Events (NE) and Impact of Dose Intensity in the Adjuvant Treatment of Breast Cancer: The UK Breast Cancer Neutropenia Audit Group.

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Indirect evidence suggests that maintaining dose intensity (DI) is important in the adjuvant chemotherapy of primary breast cancer. The strongest data are from the Milan CMF series of Bonadonna et al. where a threshold achieved DI of >85% planned was associated with better long term survival. The principal reason for dose modifications are NE, but data indicating the frequency and impact of NE on DI are limited. Prospective or retrospective data on 422 patients with Stage I-III primary breast cancer were collected from 15 UK centres. The incidence of NE (hospitalisation due to febrile neutropenia, dose delay of ≥ 1 week, or dose reduction of ≥ 15% due to neutropenia) was recorded. Various regimens were administered, essentially either CMF- or anthracycline-based (61% and 37% of patients, respectively). Only 6% of patients received G-CSF at any time. Overall, 29% of patients experienced NE, with dose delay being the most common strategy to minimise subsequent risk of neutropenic sepsis. NE had a significant impact on the ability to deliver the planned DI: in those who experienced NE, around one-third of patients received <85% DI, and 3% and 9% of patients receiving CMF- or anthracycline-based regimens, respectively, achieved <70% DI. Overall, a trend towards improved DI was seen in those neutropenic patients treated with G-CSF. Noting the ASCO guidelines on secondary prophylaxis, patients who experienced one NE were at high risk of experiencing a second event; being 56% and 72% in patients receiving CMF- and anthracycline-based regimens, respectively. In the adjuvant chemotherapy of primary breast cancer, NE are common, very likely to be repeated in an individual patient, and have a significant impact on received DI.

235 The Adjuvant Therapy of Breast Cancer: How to Translate Proportional Risk Reductions into Absolute 10-Year Benefits.

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Most measures of therapeutic efficacy in the adjuvant setting are based on proportional or relative reductions in event rates. This is not an intuitive concept and it is daunting to translate proportional reductions into clinically more meaningful absolute benefits, such as the probability of being disease-free 10 years hence. For example: How does a 25 percent relative reduction in risk of death per year with tamoxifen compare to a 35 percent relative risk reduction with chemoendocrine treatment at 10 years for a 60 year old otherwise healthy woman with 3.5 cm, hormone receptor positive, lymph-node negative breast cancer; and with an expected baseline recurrence risk of 30 percent at 10 years?

To first obtain baseline 10 year prognoses for the various presentations of patients with primary breast cancer, 11 experienced national breast cancer experts were surveyed. Despite significant variations in individual responses, means of the answers for each clinical situation provided prognoses quite similar to those determined by a prognostic tool developed by Dr Peter Ravdin (Semin Oncol 23:43-50, 1996).

Then, in an effort to calculate expected absolute benefits from adjuvant therapy in a more transparent, easily accessible manner, a computer spreadsheet was designed to provide immediate graphic and numeric output on one screen. The input parameters were kept as simple as possible. They can be varied at will on the same screen. Thus, effects due to changes in the expected efficacy of adjuvant therapy, or the estimated baseline prognosis, are visible instantly and serve as an education tool for both physicians and patients alike. From this spreadsheet, entitled "Numeracy", simplified tables were derived to allow a physician to use this information without the use of a computer.

The prognoses from this spreadsheet "Numeracy" correlate almost uniformly with prognoses from Dr Ravdin's program called "Adjuvant". Therefore, the findings from this work corroborate the findings from Dr Ravdin's work, while providing an alternative means for accessing such prognostic information for clinical practice.

236 Pharmacokinetics of 'Arimidex' and Tamoxifen Alone and in Combination in the ATAC Adjuvant Breast Cancer Trial.

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The ATAC trial evaluates in a randomized double-blind design, 'Arimidex' (anastrozole) alone (A) or in combination with tamoxifen (AT), relative to tamoxifen alone (T) as 5-year adjuvant treatment in 9,335 postmenopausal women with early breast cancer. Patients (pts) included in the pharmacokinetic (PK) sub-protocol had to have been on ATAC for ≥3 months, taking their medication in the morning and been 100% compliant for the preceding 14 days. Blood samples were collected 24±4h after last dose. Trough (Cmin) plasma concentrations of A, T and desmethyltamoxifen (DMT) were measured by validated methods. In a separate ATAC cohort of 167 pts the effect of the 3 treatments on plasma oestradiol (E2) levels was assessed in samples taken at baseline and 3 months. The PK results were based on a total of 347 pts (131 A, 111 T, 105 AT). The mean steady-state trough plasma concentrations of T and DMT were statistically equivalent in pts receiving T and AT: mean [T]=103.8ng/ml in both groups; mean [DMT]=286.6 and 293.8ng/ml in the T and AT groups, respectively. The mean A levels were 27% lower in the presence of T than with A alone. Similar findings have been reported previously for letrozole, although of a greater magnitude (mean 38%; 90% CI:32-43%, Clin Cancer Res 5, 2338-43, 1999) but the mechanism remains unknown. With respect to E2 levels, mean levels were 21.3, 19.3 and 21.6pmol/l prior to treatment and 3.7, 20.9 and 3.6pmol/l after 3 months in the A, T and AT groups, respectively. On-treatment values were below the detection limit (3pmol/l) in 43.6 and 38.5% of the A and AT groups, respectively. We conclude that (a) the lack of effect of A on T and DMT levels and (b) the observed fall in blood A levels having no significant effect on the E2 suppressive effects of A, indicate that no reduction in the efficacy of either A or T would be anticipated in the combination arm of the ATAC trial.

237 Adjuvant Chemotherapy Vs No Further Treatment in High-Risk Node Negative Breast Cancer. Ten Year Results of a Prospective Randomized Trial.

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Background: To determine the long-term impact on DFS and OS of adjuvant chemotherapy in high-risk node negative breast cancer patients, in January 1988 we implemented a prospective, double blind randomised trial, when patients were randomly allocated to receive standard CMF or no further therapy.

Subjects and Methods: From January 1988 to January 1990 a total of 584 patients were randomized to receive 8 cycles of CMF (Cyclophosphamide 600 mgs/m^2 + Methotrexate 60 mgs/m^2 + 5-Fluorouracil 750 mgs/m^2, day 1 every 21 days, 8 cycles) or no chemotherapy. To be included pt. had to display the following criteria: 1. Anatomopathogical diagnosis of infiltrating ductal carcinoma; 2. Modified radical mastectomy or a total mastectomy; 3. With negative axillary nodes; 4. Negative estrogen receptors; 5. With tumoral size ≥ 3 cm. Signed informed consent was obtained from all pt.

Results: DSF and OS at 10 years were 77 % and 89 % respectively in pt. who underwent chemotherapy, meanwhile this figures were 41 % and 52 % respectively in patients without chemotherapy ($p < 0.005$ for DFS and $p < 0.001$ for OS). No severe side effects were noted in the CMF group. No second malignancies were recorded in such subgroup of pt.

Conclusions: The appreciated 36 % in recurrence risk reduction and 37 % reduction in mortality risk after 10 years follow up, confirm the role of adjuvant chemotherapy in the management of high risk node negative breast cancer patients.

238 Feasibility of Full Dose Docetaxel (Taxotere™) after Dose-Intensive Doxorubicin (Adriamycin™) as Adjuvant Therapy for High-Risk Primary Breast Cancer.[1]

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[1] In keeping with principles of a series of phase II dose intensity studies at the University of Washington, [2] we investigated the feasibility of administering four cycles of full dose docetaxel following 12 weeks of dose-intensive doxorubicin to women (pts) with high risk primary breast cancer. Of 47 pts accrued to study, 41 are eligible (ineligible: 2 previous malignancies, 2 stage IV disease, 2 pending), with a median age of 46 (20-67). Thirty-three pts have completed treatment, 2 developed progressive disease on study, 4 stopped treatment for other reasons (1 for "naturopathic" therapy, 1 felt by provider to be at sufficiently low risk on review of prognostic factors to truncate therapy; 1 declined further treatment); 8 pts continue on treatment. Doxorubicin was planned at 28 mg/m2/week with scheduled G-CSF (NeupogenTM) support days 2-7. Three weeks from last doxorubicin dose, docetaxel was begun at 100 mg/m2 every three weeks with prophylactic ciprofloxacin for 10 days, starting day 5. Delivered dose intensity [DDI] for doxorubicin was 27.8 mg/m2/week. Thirty-four pts have completed the planned four cycles of docetaxel. The only grade 4 toxicity during the docetaxel portion of the study was neutropenia in 23 pts, with six admissions for febrile neutropenia (4% of administered cycles, 18% of pts). Grade 3 toxicities included neutropenia/leukopenia 7, infection 8, vomiting 1, ulcerative vaginitis 1, fatigue 3, myalgias/arthralgias 3, nail loss 2. With 33 evaluable patients, mean DDI of docetaxel was 37.5 mg/m2/wk (range 27.1-46.7), with 32/33 patients (97%) receiving at least 30 mg/m2/week. We conclude full dose docetaxel is well tolerated following dose-intensive administration of doxorubicin, and await outcome data in these patients.

[1]Supported by Grant-in-Aid from Rhone-Poulenc Rorer and research grant from Amgen.

[2]See, e.g., Livingston RL et al, Proc ASCO 2000, Abst 525.

239 Actually Applied Dose Intensity (DI) of Adjuvant Chemotherapy in Clinical Practice in Patients (Pts) with Primary Breast Cancer (BC).

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Dose delay (DD) and dose reduction (DR) are common features in managing toxicity of adjuvant chemotherapy (ACHT) jeopardizing the impact of treatment. This is important in Pts not treated in trials. As reported by Hryniuk (1988), outcome is directly correlated with the initial DI applied. Bonadonna (1995) reported that reduction of DI to =< 85 % of the intended CMF regimen in ACHT has no impact on treatment outcome. In a retrospective analysis 175 Pts with BC treated from 1991-99 have been evaluated for type of treatment (anthracycline based (A) or CMF), DD, DR and use of G-CSF (filgrastim) in a total of 870 cycles (cs) of ACHT. Median age was 51 yrs.; median tumor size was 2.5 cm. 57 Pts were node negative, 41 Pts had 1-3 LN+, 67 Pts had >=4 LN+. Of 175 Pts treated 110 (62%) received CMF and 65 (38%) received A. 73% of Pts 1-3 LN+ received CMF and 27% A. Pts with >=4 LN+ 38.8% received CMF and 61.2% received A. DR > 15% was present in 2.3% of cs. Reduction of DI was due to DD, ranging from 7-98 days. DD was more frequent with CMF (13.5%) compared to A (8.4%), (p<0,01). The overall probability of DD was 11.5% (100/870 cs): 13.5% for CMF (71/535 cs) and 8.4% (28/335) for A. This led to =<85% of the intended relative total dose intensity (RTDI) in 12% of the Pts treated with 6 cs of CMF and 10-12% in Pts treated with 4 to 6 cs of A. G-CSF support in CMF: 26/535 cs and 39/335 cs for A. G-CSF use was limited to one day only 16/26 cs (61%) with CMF and 17/39 cs (44%) with A. In less than 10% of the cs G-CSF was given according to the ASCO-guidelines (1998) for the intended use of G-CSF. Conclusion: Our data suggest that the clinical management of ACHT (CMF or A) is associated with reduction of RTDI (=< 85%) of 18.7% (after 4 cs) and 15.6% (after 6 cs). It is of special interest that RTDI [=<]85% is more frequent in CMF (24.3%/4 cs; 15.4%/6 cs) than in A (12.1%/4 cs ;15.6%/6 cs). We propose that the use of G-CSF according to the ASCO-guidelines would have been able to avoid at least the hematotoxicity related treatment delays, therefore offering the chance to deliver the planned dose of chemotherapy on time.

240 Adriamycin-Cyclophosphamide (AC) Adjuvant Chemotherapy Produced a 90% 5 Year Disease-Free Survival (DFS) in 450 Women with Rapidly Growing Invasive Node Negative Breast Cancer.

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In 1988 we initiated a prospective clinical trial to evaluate the effect of adjuvant chemotherapy with AC chemotherapy in women with invasive node-negative breast cancer at increased risk of recurrence based on rapid proliferation rates (S phase fraction [SPF] >6.7% in 80% of cases or elevated Ki67/MIB1 >20%). Based on the work of Clark et al (NEJM 320_627, 1989) the predicted 5 year DFS without adjuvant chemotherapy was 70% for this group. Between 1988 and 1998, 450 women received either 3 courses of A (40 mgs/m^2 IV on d1) and C (800 mgs po over 4 d or 600 mgs/m^2 IV on d1) for T_1N_0 and 6 courses with T_2N_0 cancers. Tamoxifen (20 mgs) was given for 5 years for postmenopausal women with receptor-positive cancers. At a median follow-up of 55 months, 5 year DFS for the population, mean age 50 (26-77) is shown:

Tumor Size	No.	DFS	OS
All cases	450	90±2%	94±1%
T_1N_0	276	90±2%	96±1%
T_2N_0	174	91±2%	92±2%

Conclusion: Adjuvant chemotherapy with AC (3 courses for T_1N_0 and 6 courses for T_2N_0) plus tamoxifen is effective in improving the overall DFS for women at high risk with invasive breast cancer and increased rates of proliferation determined by SPF or histochemistry.

241 Suboptimal Dosing in Adjuvant Breast Cancer Chemotherapy: Evidence from a Nationwide Survey.

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Recent trials have shown that receiving less than the standard dose of adjuvant chemotherapy is associated with poorer outcomes, strongly suggesting a threshold effect for optimal chemotherapy dosing in adjuvant breast cancer treatment. To characterize chemotherapy dosing in clinical practice, we reviewed data from a nationwide survey of 1243 community oncology practices, conducted between 8/1/97 and 4/30/00. The primary objective was to determine practice patterns with respect to relative dose intensity (RDI) over a course of conventional adjuvant breast cancer chemotherapy. The frequency of treatment delays, dose reductions, and episodes of febrile neutropenia were also studied. Patients were stratified according to age (mean 51.9 yrs), weight, height, menopausal status (48.8% postmenopausal), nodal status (54.0% positive), and estrogen receptor status (56.3% positive). A total of 20,799 records were analyzed. The overall results demonstrated that 18.3% of patients received a RDI of <85%, 25.4% experienced dose reductions, 43.1% had a treatment delay, and 6.0% had an episode of febrile neutropenia. Patients ≥65 years (16.4%) had a consistently poorer quality of chemotherapy delivery compared to younger patients, including a greater number of dose reductions (31.1% vs 24.7%, p<.001), treatment delays (50.4% vs 41.7%, p<.001), and RDI <85% (24.8% vs 17.5%, p<.001). CMF (cyclophosphamide/methotrexate/5-fluorouracil) was the most common regimen administered (43.6%), followed by AC (adriamycin/cyclophosphamide; 33.0%), and CAF (cyclophosphamide/ adriamycin/5-fluorouracil; 19.5%). A RDI <85% was more likely with CMF (22.2%) and CAF (24.4%) than AC (10.7%) (p<.001). Similar differences between regimens were observed for dose reductions and treatment delays. Patients with negative nodes were more likely to receive <85% of targeted dose intensity (45.9%) than patients with 1-3 nodes (32.1%) or ≥4 nodes (21.9%) (p<.001). Multivariate logistic regression analysis was performed for each major outcome, including all surveyed variables. The results of additional analyses to identify individual patient risk factors for neutropenia and suboptimal chemotherapy delivery will be presented, as well as findings from a follow-up survey currently underway to incorporate emerging trends in chemotherapy and to further characterize reasons for suboptimal chemotherapy delivery. (Supported by a grant from Amgen Inc.)

242 Adjuvant FEC Polychemotherapy for Fast Proliferating Node Negative Breast Cancer Patients: A Randomized Clinical Trial.

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Since January 1990, we consecutively analyzed in a prospective series of node negative breast cancer women, the primary tumour proliferative activity to individualize subgroups of patients with worse prognosis candidated to adjuvant therapy. Tumour proliferative activity was determined according to 3H-Thymidine incorporation assay and authoradiographic technics.

Women with fast proliferating breast cancer (3H-Thymidine Labeling Index, TLI>2.3%) were randomized to receive: arm a) 6 cycles of adjuvant polichemotherapy according to FEC schedule; arm b) no therapy until disease progression.

125 and 123 patients treated with radical surgery for pT1-2N0M0 breast cancer were randomized in FEC and Control arm respectively. After 70 mo. of median follow-up, 27(21.6%) and 39 (32.2%) events were observed in FEC and Control arms repsectively with a significant reduction of loco-regional relapses in FEC group (3 vs 12;p<0.02).

A 5-yrs DFS of 81% (95%CI:88-74)for FEC treated women with respect to 69% (95%CI:75-63) of the Control group was observed (p<0.03 by log rank test). The analysis of DFS according to Cox multivariate test confirmed that the impact of FEC therapy on prognosis is independent from oestrogen receptor status and tumour size (HR for the untreated group=1.72; 95%CI:1.1-2-3).

An adjuvant polichemotherapy according to FEC schedule seems able to significantly improve the prognosis of fast proliferating node negative breast cancer patients.

Project partially supported by Ministry of Health-Italian Gouvernement and National Council of Research, CNR Italy.

243 Determinants of Outcome in Adjuvant Chemotherapy of Breast Cancer: Dose Intensity Vs. Total Dose Vs. Dose Size.

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Early trials testing dose intensity appeared to corroborate its importance but are inconclusive because dose size and cumulative dose were usually increased concurrently. To assess their contributions we developed scales based on a knowledge of the dose intensity of each drug required to produce a 30% CR+PR rate in first line therapy of breast cancer (the Unit Dose Intensity or UDI). The effective dose intensity of a drug combination can then be determined by dividing the dose intensity of each constituent drug by its UDI and adding the fractions together to yield the *Summation Dose Intensity (SDI)*. The effective total dose is derived by multiplying the combination's SDI by the treatment duration in weeks to yield the *Dose Intensity Product or DIP* (1 DIP unit = 1 SDI unit x 1 week). The effective dose size of a combination is derived by dividing the individual drug dose sizes by their UDI's and adding the fractions together to yield the *Unit Dose or UD.*

The relative contributions to chemotherapy outcome of SDI, DIP and UD can then be determined. Using these parameters ten randomized trials with 18 arms testing dose intensification in adjuvant chemotherapy of breast cancer with at least 3 years of follow-up were analysed. The relationship was assessed between relapse-free survival (RFS) and differences in the three dosage parameters. **Results:** No independent relationship was detected between differences in UD's and RFS. However, despite variations in nodal groups treated and duration of follow-up, there was a 3 way relationship between RFS, DIP and SDI. If the difference between DIP's of the trial arms was greater than 7 units, regardless of differences in SDI's the trials were positive. If the differences in DIP's was 7 or less but there was a difference greater than .60 SDI units between arms, one trial was positive and 3 showed subgoup differences. If the differences in DIP's was less then 7 units and the differences in SDI less than .60 units, 5 trials were negative and one showed subgroup differences. Conclusions from this analysis must be tentative because a plot indicates a lack of data from trials (pending) testing large differences in SDI with no change in DIP. With that caveat, differences in DIP (SDI x treatment duration in weeks) appeared to be the major determinant of improved RFS.

244 Improved Survival with Continuous Infusional ECisF over Conventional AC as Pre-Operative Chemotherapy for Early Breast Cancer.

Smith IE, A'Hern RP, Howell A, Hickish T, O'Brien M, Mansi J, Wilson C, Robinson A, Pratt W, Price C, Perren T, Laing R, Jones A, Iveson T, Stein R, Gallagher C, Morgan J, on Behalf of TOPIC Trial Group. Royal Marsden, United Kingdom.

Preoperative continuous infusional (ci) ECisF (E 60mg/m^2 iv bolus, Cis 60mg/m^2 iv, both x 3 weekly (wk) x 6 courses, with ci 5FU 200mg/m^2 x 24 hourly x 18wk by ambulatory pump), highly active in early breast cancer (98% response), has been compared with conventional AC (Adriamycin 60mg/m^2, Cyclophosphamide 600mg/m^2 both iv bolus x 3 wk x 6 courses) in a Phase III multicentre randomised trial (TOPIC: Trial of Preoperative Infusional Chemotherapy), involving 426 pts with needle biopsy proven invasive operable ≥3cm breast cancer, followed by appropriate local surgery ± radiotherapy and tamoxifen 20mg daily x 2-5 years. Patient characteristics for ci ECisF (211 pts) v AC (215 pts) respectively were as follows: median age 46 (range 22-68) v 47 (25-66) yrs, premenopausal 64% v 66%, median tumour diameter 5 (3-11) v 5 (3-15)cm. Results at median follow-up of 30 months are as follows:

	ci ECisF	AC	
Complete Remission	34%	31%	p=0.53
Overall Response	77%	75%	p=0.56
3-yr Mastectomy Rate	37%	43%	p=0.13
3-yr Relapse Free Survival	77%	66%	HR 0.77 (0.52-1.14) p=0.19
3-yr Overall Survival	90%	80%	HR 0.56 (0.32-0.97) p=0.04
path CR rate (subset of 250 pts)	16%	16%	
path CR + DCIS alone	26%	28%	

Grade 3/4 toxicity was low in both arms but significantly worse for ECisF for nausea/vomiting, diarrhoea, thrombosis and palmar erythema. This interim analysis shows a significant survival benefit for continuous infusional 5FU-containing chemotherapy over conventional AC, not predicted for by clinical or path response rates. Biological predictive studies are underway.

245 Study of Differential Gene Expression before and after Preoperative Chemotherapy (PChT) of Breast Cancer.

Volz JO, Frühauf JH, Vielhauer S, Schneider J, Volz-Köster SR. Obstetrics and Gynecology, University Hospital Mannheim, Mannheim, Germany.

Introduction: Resistance to chemotherapy frequently poses a problem in the treatment of breast cancer. Chemoresistance is mostly associated with treatment failure, as its diagnosis is based on clinical detection of recurrent and/or progressive disease. Preoperative chemotherapy (PChT) offers the possibility to monitor tumor response after exposure to cytotoxic agents in vivo.

Patients and methods: Thirty Patients with T2-4, N0-1, M0 tumors were examined. Tumor tissues were obtained using sonographically guided core biopsy prior to treatment. Tissues were snap-frozen and stored at -70°C until processing. PChT consisted of 4 cycles of Adriamycin (50mg/m^2)/Docetaxel (75mg/m^2) with G-CSF support q 2 weeks. A second tumor sample was obtained at the time of operation. Isolation and purification of mRNA was performed using the Trizol® and Dynabeads® methods. After transcription of mRNA (rtPCR) and radioactive labelling, differential gene expression analysis was performed using a cDNA array carrying 30.000 genes.

Results: All patients showed primary response to PChT. From all biopsies a sufficient amount of mRNA was obtained for reproducible hybridization. The tissue obtained after PChT was insufficient for preparation in 8 cases due to extensive tumor regression. The remaining 22 samples showed different gene expression patterns compared to the corresponding biopsies. A number of new genes so far not known to be involved in cytotoxic-induced mechanisms have been identified.

Conclusion: The knowledge of changes in gene expression patterns after chemotherapy allows a better understanding of the molecular mechanisms involved. The characterization of new genes will enable us to develop a specialized breast cancer array with those genes found to be relevant for response and resistance to chemotherapy. This will result in individualized therapy decisions based on the knowledge of molecular properties in individual cancer patients.

246 Remission Rate and Toxicity of Preoperative Therapy with Concurrent Application of Tamoxifen Together with Adriamycin and Docetaxel in Comparison to Chemotherapy Alone – Results of the Phase IIB - GEPARDO Trial.

Blohmer JU, von Minckwitz G, Costa S, Raab GH, Eidtmann H, Hilfrich J, Jackisch C, Merkle E, Gademann G, Graf E, Tulusan AH, Kaufmann M, and the GEPARDO Study Group. GABG, Berlin, Germany.

Does the concurrent application of Tamoxifen influence the remission and toxicity of a preoperative chemotherapy in breast cancer patients?

250 patients from 56 clinics with a histologically estimated unilateral bidimensional measurable operable breast cancer (T > 3cm, N0-2, M0) were randomized to receive preoperative 4 cycles of adriamycin (50 mg/m2) and docetaxel (75 mg/m2) q 14 days including G-CSF support from day 6-10 (ADoc) with or without tamoxifen (Tam, 30 mg/day) independent from receptor status. Tumor size and shrinking was assessed by palpation, mammography and ultrasound. Estrogen receptor was positive in 59 % of the pts (preoperative assessment in 196 pts). Patient characteristics were well balanced between the two treatment arms (Raab G et al. ASCO 2000, #322).

The rate of pCR was 9.1% with ADoc + Tam and 10.3% with ADoc (95%-CI for difference: -8.6%, 6.2%). The difference between the 2 arms of the study was not significant. Compliance: 972 of the planned 992 (98%) cycles of chemotherapy were given. 19 cycles were not given after a therapy related toxicity of grade 4 (according to the study protocol). In one cycle the chemotherapy dose was reduced (personnel decision of the doctor). In only 3.2% of the cycles the biweekly interval was longer than 16 d. Toxicity (Grade 3 or 4 in % of cycles, ADoc + Tam vs. ADoc): Anemia: 2.5 vs. 2.5; neutropenia: 31.7 vs. 22.4; thrombocytopenia: 1.7 vs. 0; nausea: 3.3 vs. 5.7, diarrhea: 2.5 vs. 4.0; mucositis: 4.2 vs. 3.2; allergy: both 0.8, skin nail changes: 6.7 vs. 2.4; infection: 6.7 vs. 1.6; peripheral neuropathy: 5.0 vs. 3.2; fluid retention: both 0; deep vein thrombosis: 4 pts vs. 0 pts.

Although the preoperative therapy was given over a short period of 8 weeks, the rate of pathological complete remission was comparable with the pCR of other studies. There was no benefit of concurrent preoperative hormone- and chemotherapy in terms of remission rates and toxicity.

247 High Complete Pathological Response in Locally Advanced Breast Cancer Using Paclitaxel and Cisplatin.

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Background: In an earlier study, a high response rate in metastatic breast cancer using paclitaxel (P) and cisplatin (C) was demonstrated. A phase II study using the same regimen (PC) has been conducted in locally advanced breast cancer (LABC).

Patients & Methods: A total of 72 consecutive patients with non-inflammatory LABC (T2 ≥ 4 cm, T3 or T4, N0-N2, MO). Patients were scheduled to receive 3-4 cycles of the neoadjuvant PC (paclitaxel 135 mg/m^2 and cisplatin 75 mg/m^2 on day 1) every 21 days. Patients were then subjected to surgery and subsequently received 6 cycles of FAC (5-fluorouracil 500 mg/m^2, doxorubicin 50 mg/m^2, and cyclophosphamide 500 mg/m^2) or 4 cycles of AC (doxorubicin 60 mg/m^2, and cyclophosphamide 600 mg/m^2). Patients then received radiation therapy, and those with hormone receptor positive tumors were given adjuvant tamoxifen intended for 5 years.

Results: The median age was 39 years. Clinically, 7%, 58%, and 35% of patients had T2 ≥ 4 cm, T3, and T4, respectively. The clinical nodal status was N0, N1, and N2 in 38%, 40%, and 22% of patients, respectively. Disease stage at diagnosis was IIB (33%), IIIA (27%), and IIIB (40%). Complete and partial clinical response to PC was demonstrated in 13 (18%), and 52 (72%) of patients, respectively, for an overall response of 90% (95% confidence interval [CI], 79% to 100%). Modified radical mastectomy was performed in 72%, conservative surgery in 24%, while 4% refused surgery. Of those patients with evaluable pathologic response (68 patients), complete pathologic response (pCR), and partial pathologic response was achieved in 15 (22%) and 51 (75%) of patients, respectively, for an overall response of 97% (95% CI, 95% to 99%). At a median follow-up of 22 (± 3.5) months, 58 (81%) were alive with no recurrence, 9 (12%) were alive with evidence of disease, and 5 (7%) were dead. Of the 68 patients with complete pathologic data, 12 developed recurrence (18%). None of the patients achieving pCR have relapsed. The median overall survival has not been reached for all 72 patients with a projected 3-year survival (± SE) of 90% (± 4%). On the other hand, the median progression-free survival (PFS) was 42.1 (± 4.8) months with a projected PFS of 74% ± 7% at 3-years (for 68 patients). PC was shown to be safe.

Conclusions: PC regimen as used in LABC produced a high pCR and was associated with favorable survival outcome.

248 GEPARDUO - A Prospective Randomized Trial on Preoperative Chemotherapy (CHT) in Operable Breast Cancer (T2-3,N0-2,M0) Comparing Dose Intensified (DI) Adriamcycin/Docetaxel (ADOC) with Sequential Adriamycin/Cyclophosphamide Followed by Docetaxel(AC-DOC) - First Interim Analysis.

Jackisch C,[1] von Minckwitz G,[2] Eidtmann H,[3] Costa SD,[2] Raab G,[4] Blohmer JU,[5] Schuette M,[6] Gerber B,[7] Merkle E,[8] Gademann G,[9] Lampe W,[10] Hilfrich J,[11] Tulusan AH,[12] Graf E,[13] Kaufmann M.[2] [1]Obstetrics and Gynecology, University of Muenster, Muenster, Germany; [2]Department of Obsterics and Gynecology, University of Frankfurt/Main, Frankfurt/Main, Germany; [3]Department of Obstetrics and Gynecology, University of Kiel, Kiel, Germany; [4]Department of Obsterics and Gynecology, Frauenklinik vom Roten Kreuz, Munich, Germany; [5]Department of Obsterics and Gynecology, University Berlin Charité, Berlin, Germany; [6]Obstetrics and Gynecology, Bethesda Hospital, Essen, Germany; [7]Obstetrics and Gynecology, University of Rostock, Rostock, Germany; [8]Obstetrics and Gynecology, University of Halle, Halle, Germany.

The German Adjuvant Breast Cancer Group (GABG) previously demonstrated that neoadjuvant DI CHT (ADOC q14dx4 +/- Tamoxifen) results in a pCR-rate of 9.7% (Raab et al. ASCO,2000). In a subsequent protocol (n=1.000 pts./3yrs.) we would like to increase the pCR-rate by comparing a dose-intensified schedule (ADOC: adriamycin 50mg/m^2 + docetaxel 75 mg/m^2 q 14dx4 + G-CSF) to a sequential schedule (AC-DOC: adriamycin 60 mg/m^2 + cyclophosphamide 600 mg/m^2 q 21dx4 followed by docetaxel 100 mg/m^2 q21dx4) prior to surgery. Tamoxifen (20 mg/d for 5 yrs.) starts in both arms with CHT. Within 11 months 317/1000 pts. entered this trial: 153 pts. (51.5%) (ADOC),144 pts. (48.5%) (AC-DOC). Median age was 52 yrs. Tumor assessment by physical examination results in median tumor size of 4 cm (CI: 3-5), by best imaging 2.9 cm prior CHT. Nodal status prior CHT: N0, 176 (94 ADOC, 82 AC-DOC), N1, 116 (57 ADOC, 59 AC-DOC), N2, 5 (2 ADOC, 3 AC-DOC). Data on toxicity/pts. are availible for 79 pts. Grade 3 / 4 hematologic toxicity: anemia 0% neutropenia: ADOC: 25%, AC 53%, DOC 37.5%, thrombopenia: 0%. Grade 3 / 4. Non-hematologic toxicity: alopecia: ADOC: 80%, AC 91%, DOC 70%, nausea: ADOC: 0%, AC 6%, DOC 0%, skin: ADOC: 0%, AC 0%, DOC 10%, nail: ADOC: 3%, AC 0%, DOC 20%, neurotoxicity: ADOC: 0%, AC 0%, DOC 0%, hot flushes: ADOC: 3%, AC 3%, DOC 0%, Constipation: ADOC: 0%, AC 6%, DOC 0%, No severe infections, fluid retention, diarrhea, or allergic reactions occured.

8/43 (18.6%) patients a pCR was documented. Both schedules are feasable, well tolerated, and highly effective as preoperative CHT in primary operable breast cancer.

249 Relationship of Clinical and Pathologic Response to Neoadjuvant Chemotherapy and Outcome of Locally Advanced Breast Cancer.

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Clinical response of locally advanced breast cancer (LABC) to neoadjuvant chemotherapy presumably reflects what is occurring with distant micrometastases. Since pretreatment histologic characteristics of these tumors predict response to chemotherapy, it may be possible to predict outcome by studying pretreatment histologic characteristics of the primary tumors. Specimens from 144 patients with LABC were examined before administration of neoadjuvant chemotherapy and after surgery. Complete clinical response with no palpable tumor was noted in seven patients (8%) and complete pathologic response was achieved in 18 patients (13%). Both clinical (P=0.058) and pathologic response (p=0.011) were related to tumor size at the time of diagnosis: smaller tumors were significantly more likely to respond to chemotherapy than larger tumors. Histologic evidence of chemotherapeutic effect, cytoplasmic vacuolization, change in the number of mitoses and fibrosis in lymph nodes did not correlate with clinical or pathological response. Clinical and pathologic response could not be predicted by age, histology, differentiation, or type of chemotherapy. Clinical stage and pathologic lymph node status were predictive of distant disease-free and overall survival. After consideration for pathologic lymph node status no other variable was significantly related to distant disease-free or overall survival in multivariate analysis. No residual tumor was found in the axillary nodes of 27 percent (37) of the patients. Age (P=0.008), pathologic tumor size (P=0.058) and clinical lymph node status (P=0.048) were significantly related to pathologic axillary lymph node status. After consideration for age (P=0.008) and clinical lymph node status (P=0.052) no other variable was significantly related to pathologic lymph node status. Our study of patients treated with neoadjuvant chemotherapy for locally advanced breast cancers found little evidence that measurable clinical or pathologic changes attributable to chemotherapy predicted outcome. Axillary lymph node status predicted by young age, clinical nodal status and pathologic tumor size is the most important prognostic indicator in these patients. This study does not support the hypothesis that response to treatment predicts outcome after consideration for extent of disease.

250 Phase II Trial Combining Docetaxel (D) and Doxorubicine (DOX) in the Neoadjuvant Setting in Patients (PTS) with Operable Breast Carcinoma (BC): Final Results.

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D and Dox have proven their efficacy in advanced BC (response rate: 57 to 76%) with a good safety profile. In a phase III randomized study (ASCO 99-Abst n°485) the combination of these 2 agents shows an advantage versus Dox/cyclophosphamide in terms of response rate and time to progression. It was decided to evaluate the efficacy of this combination in the neoadjuvant setting. **Primary endpoint:** pathological complete response rates (pCR). **Secondary endpoint:** clinical and radiological responses rates, conservative surgery rate, relapse free survival and safety profile. **Pt selection:** histologically proven breast cancer, unilateral, non inflammatory, T2, T3, M0 tumors, breast conservative surgery, adequate hematological, renal, liver and cardiac functions, WHOPS≤ 1. **Treatment:** maximum 6 cycles every 3 weeks Dox 50 mg/m² IV immediately followed by D 75mg/m² IV. **Pt characteristics:** on 48 pts included: median age: 48 (31-63), T2:28 pts, T3:20 pts. 273 cycles (cy) administered, median No of cy: 6 (1-6). **Safety**: the most frequent grade 4 toxicities were(%pt/%cy) neutropenia (52/16), febrile neutropenia (19/4) secondary prevented by use of G-CSF. No clinical cardiac toxicity was observed. **Efficacy**: to date 47 pts are evaluable for clinical response: 15% CR, 70% PR (ORR: 84%), 33 pts for radiological response (mammography): 12% CR, 39% PR and 46 pts for pCR according to the investigator's center pCR: 13% including in situ alone (N-), conservative surgery rate: 32 pts. The combination of D and Dox is promising in the neoadjuvant setting. An independent blinded review of pathological response both with Chevallier and Sataloff Classification is ongoing and the results will be presented during the meeting.

251 High Pathological Response Rate Induced by Primary Docetaxel Monotherapy in Operable Breast Cancer.

Chollet P,[1] Amat S,[1] Penault-Llorca F,[1] Fétissof F,[2] Body G,[2] Mouret-Reynier MA,[1] Bons JM,[1] Curé H,[1] Dauplat J,[1] Bougnoux P.[2] [1]Centre Jean Perrin and INSERM U484, Clermont-Ferrand; [2]CHU Bretonneau, Tours, France.

To assess the activity and toxicity of docetaxel, 84 women with operable nonmetastatic breast cancer (stage II-III) were included in a phase II trial from September 1997. Among 84 patients (pts) who were treated by 6 cycles of docetaxel at 100 mg/m² 3 weekly, 79 are now fully assessable. Median age was 47 years (29-66), 47% were premenopausal, 69% stage II, 31% stage III breast cancer with at least another adverse prognostic factor: 40 N1, 37 SBR grade III, 39 aneuploidy and 25 both negative hormonal receptors. At initial staging, 42 pts underwent fine needle biopsy and node cytology (FN) and 37 had surgical biopsy and axillary dissection (B&AxD). Pathological proof of invasive cancer showed 62 ductal, 12 lobular and 5 other carcinomas. Results: 67 pts received full planned treatment without dose reduction or dose delay. 6 pts went out of study due to early progression and 3 pts went out of study due to toxic adverse event (acute allergic syndrome). After 6 cycles, the median size of the primary tumor decreased significantly, from 50 mm (20-130) to 10 mm (0-90) on physical examination ($p<10^{-7}$). The overall clinical response rate, evaluated by clinical, mammographic, ultrasound and/or MRI examination, was 64.1% [53.5; 74.7%], with 15% of clinical complete remissions. 24.2% of pathological complete responses (pCR) according to Chevallier classification (*Am J Clin Oncol* 1993, 16: 223) are reported in 62 pts operated at present time. Interestingly, some pCRs were noted among partial responses. There was no significant difference in both groups (FN or B&AxD, p>0.5). Conclusion: The docetaxel regimen resulted in a promising clinical complete response rate allowing a 73% of conservative surgery rate with around one third of responders in pCR (28%). This single agent therapy seems at least equivalent to the polychemotherapy already published, with a good tolerability, eventhough without direct comparison. However, the impact on patient TTO relapse has to be confirmed in a randomised studies.

252 Phase II Study of Doxorubicin and Docetaxel as Neoadjuvant Therapy for Women with Stage IIB or III Breast Cancer.

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Women with locally advanced breast cancer (LABC) have a poor long term outcome. The use of neoadjuvant chemotherapy for the treatment of women with LABC may render women with previously unresectable tumors to be candidates for definitive local therapy. Furthermore, findings at the time of surgery predict for long term outcome. Docetaxel has been shown to be active in women with anthracycline resistant breast cancer. The combination of docetaxel and doxorubicin has higher activity than a combination of doxorubicin and cyclophosphamide. We reasoned that the combination of docetaxel and doxorubicin would be an ideal combination for the treatment of women with LABC. 24 women with stage IIB or stage III breast cancer (21 evaluable for this report) were treated with four cycles of docetaxel (75 mg/m2) and doxorubicin (50 mg/m2) administered every three weeks for four cycles without the adminstration of filgrastim. The 21 women include: stage IIB-5 patients, stage IIIA-8 patients and stage IIIB-8 patients. 7 patients had T4 tumors, 10 patients had T3 tumors and 4 patients had T2 tumors. All but 4 patients had clinically evident axillary lymph nodes. Ninetenn of 21patients (90%) had at least a partial clinical response. Six patients had a complete clinical response and 4 additional patients had a palpable abnormality (PNM) that was no longer measurable (50% with CR or PNM). Two patients had progressive disease. One of 20 patients had a complete pathologic response. Sixteen of 20 patients (80%) had a reduction in tumor stage at pathologic evaluation. Ten of 20 patients had tumors of 2 cm or less on pathological evaluation. Chemotherapy was well tolerated with the major side effect being neutropenia. Grade 3/4 neutropenia occurred 100% of the time during the preoperative chemotherapy. Nine of 21 patients (43%) developed fever with neutropenia. Eleven of 21 patients (52%) required the use of filgrastim either because of fever and neutropenia or to avoid treatment delay. One patient with a history of diabetes required a 25% dose reduction of docetaxel alone because of grade 3 neuropathy. We conclude that the combination of docetaxel and doxorubicin is a highly active regime that can be safely administered to women with stage IIB/III breast cancer. Response rates for this group of patients with locally advanced breast cancer exceed those usually associated with neoadjuvant chemotherapy.

253 Phase II Trial of Neoadjuvant Chemotherapy with Docetaxel and Doxorubicin, Surgery, Adjuvant CMF, and Radiotherapy +/- Tamoxifen in Locally Advanced Breast Cancer.

Valero V, Esteva FJ, Sahin AA, Booser DJ, Strom EA, Esparza-Guerra LT, Ross MI, Rosales MF, Ibrahim NK, Cristofanilli M, Buchholz TA, Hunt KK, Hortobagyi GN. U. T. M. D. Anderson Cancer Center, Houston, TX.

Background: Docetaxel plus doxorubicin (DD) is among the most active combinations for the treatment of breast cancer (BC). This combination has shown a higher objective response rate than doxorubicin and cyclophosphamide without any significant cardiotoxicity. We designed a phase II study of this combination to determine the efficacy of this regimen as neoadjuvant chemotherapy for patients (pts) with locally advanced BC. Our objectives were to evaluate efficacy by clinical response, pathological complete response (pCR), and downstaging. Results: To date, 81 pts (target accrual 88) have been enrolled. Pts received doxorubicin 60 mg/m^2 over 15 minutes, followed 1 h later by docetaxel 60 mg/m^2. A high incidence of neutropenic fever was noted on the first 11 pts. Subsequently, prophylactic GM-CSF was added (150 mcg/m^2 SQ daily for 10-14 days). Results are available for 70 pts. Median age was 50 yrs (range 27-67). Estrogen receptor status: 24 ER+ (34%); 43 ER- (62%); 3 ER unknown (4%). Sixteen pts had stage IIIA, 1 stage IIB, 34 stage IIIB, and 19 stage IV (regional, ipsilateral supraclavicular node metastasis). Efficacy: Forty-nine pts received 4 cycles of DD, and 7 pts received 6 cycles. Five pts were not operable after 4 cycles and were switched to CMF preoperatively. Intent-to-treat clinical response rate was 90% (PR 85.7%, CR 4.3%). Three pts had no change, and 4 pts had progressive disease. Fifty-eight pts had surgery; 49 underwent modified radical mastectomy, and 9 had breast conservation surgery. Forty-four pts (63%) were downstaged. Seven pts had pCR (10%). Lymph node (LN) status after primary chemotherapy: 8 LN-; 12 had 1-3 +LN; 13 had 4-9 +LN, and 9 had 10 or more +LN. Five pts developed distant metastases (brain 3, liver 1, lung 1). Safety profile: A total of 246 courses were given. There were no unexpected toxicities and no toxic death. Twenty pts developed neutropenic fever (8 pts in pts 1-11; 12 in pts 12-70); 8 serious infection, 6 mucositis grade ≥ 3, 11 significant fatigue, 3 significant myalgias, and 2 colitis. Conclusion: These preliminary results indicate that DD is a highly effective regimen for stage III/IV (regional) BC. GM-CSF reduced the incidence of neutropenic-related events.

254 Neoadjuvant Chemotherapy with Taxotere-Epirubicin-5-Fluorouracil (TEF) in Locoregionally Advanced Breast Cancer: Preliminary Report.

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Taxans are being used currently as an adjuvant treatment for early breast cancer and as a neoadjuvant treatment for locoregionally advanced breast cancer after its efficacy was shown in treatment of metastatic breast cancer. Sixtythree patients with locoregionally advanced breast cancer patients were recruited. Three patients were excluded from study before the evaluation of response. Median age of the patients were 50 (range: 25-77). Twentyseven and thirtythree patients were premenopausal and postmenopausal respectively, patients received four cycles of Taxotere 80 mg/m2, Epirubicin 60 mg/m2 and 5-Fluorouracil 500 mg/m2, repeated in every 21 days. Following completion of four cycles of chemotherapy, appropriate surgery was performed. After the surgery, patients received one cycle of TEF chemotherapy regimen and following chemotherapy, radiotherapy was applied and at the end two more cycles of TEF chemotherapy regimen were introduced. Complete response and partial response were observed in 15 (25%) and 42 (70%) of the patients respectively following four cycles of TEF chemotherapy regimen preoperatively. Total response was 95% and only 3 patients primary lesion (5%) progressed. The mean disease free survival was 15.9-6.8 (3.5-28) months and the mean overall survival was 18.6-7.2 (5-30) months. Most frequent side effects were nausea-vomitting, mucositis, alopecia and leukopenia. Atrial fibrilation as a cardiotoxicity was observed in one patient. Ejection fraction was decreased more than 10% in 2 patients during the treatment.

255 Phase II Trial of Modified Sequential CAF Regimen in Locally Advanced Breast Carcinoma (LABC): Safety and Efficacy Report.

Valdivia S, Santillana S, Cotrina J, Gomez H, Abugattas J, Velarde R, Vigil C, Leon L, Flores C, Vallejos C. Instituto de Enfermedades Neoplasicas, Lima, Peru.

Background:Treatment of LABC requires more efficient primary systemic treatments to improve not only the response rates but also overall survival. We evaluated the role of sequential administration of chemotherapy (as shown by Bonadonna) in LABC pts.

Objectives:To test the efficacy and safety of a intensified sequential modification of CAF regimen in previously untreated locally advanced breast cancer pts, defined as: DOXORUBICIN 90 mg/sqm/q3 weeks x 3 courses, followed by 3 courses of a combination of CYCLOPHOSPHAMIDE 1 gr/ sqm x 1 plus 5FLUORURACIL 1 gr/sqm/day x 3 (2 hours IV IF).) (A/ CF-FU).

Methods:We performed a Phase II trial to evaluate the A/ CF-FU regimen . Inclusion criteria included stage III-A(14%) and III-B(86%)LABC with measurable disease., performance status < 2, no prior systemic therapy was allowed. No growth factors were used.From 1/1996-4/99, 85 pts. were admitted , median age 44 years(range 25-75),with 50% of premenopausal pts.After completing chemotherapy ,local treatment was performed including radical or conservative surgery when indicated or locoregional radiotherapy.We present toxicity and efficacy data.

Results: An intention to treat analysis was done for response rate (n:85) and relative dose intensity(RDI)analysis was done in 77 pts. Results are:

(N:85 PTS)	CR (%)	PR (%)	RR(%)	RDI(n:77)
ADRIA 90	12(14.1)	52(61.2)	64(75.3)	0.85
CF+5FU	33(38.8)	36(42.4)	69(81.2)	0.82
A/ CF-FU (CI 95%)	38.8 %(28.4-49.2)	424%(32,0-52,7)	81.0%(72,1-89,3)	0.83

41/69(48.3%) pts. underwent surgery (radical:31 and conservative 10) and 38 received radiotherapy. Pathological complete response was 9%.Toxicity was moderate with 25% of grade 3-4 myelosupression and only 5% of grade 3-4 gastrointestinal toxicity. No treatment related deaths were reported. With a median follow up of 18,7 months, the 2 year -progression free survival(2y-PFS) is 80.4% and 2 year -overall survival(2y-OS) is 85.0%.

Conclusions: Sequential A/ CF-FU is an effective and well tolerated regimen for LABC.Our preliminary data suggest that the intensified sequential combination of CAF is superior in terms of complete response rates than conventional CAF. Further follow up will determine the impact of this strategy to improve the outcome on LABC pts.

256 Dose-Dense Preoperative Chemotherapy with Sequential Doxorubicin (D) and Docetaxel (T) for Operable and Inoperable Stage II-IIIB Breast Cancer.

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New strategies for locally advanced breast cancer (LABC) including primary chemotherapy may provide improved local tumor control, increased breast conservation, and earlier treatment of systemic micro-metastatic disease. A prospective study was designed using rapid sequential delivery of D 75 mg/m2 q2wks X 3 cycles followed by T 100 mg/m2 q2wks X 3 cycles to assess the efficacy and toxicity of this regimen in pts with LABC. Objectives included evaluation of clinical and pathologic response and investigation of perioperative circulating tumor cells (CTC). CTC were detected using a sensitive tumor enrichment assay with anti-epithelial antibody selection and subsequent immunostaining capable of detecting 1 tumor cells in 1X106 blood. 26 pts of a planned total of 35 have been enrolled. 18 pts are clinically evaluable (2 off study due to toxicity and 6 still on treatment) and 16 have undergone surgery. Median age is 47, stage of disease T2 (5 pts), T3 (5 pts), T4 (8 pts), N0-2. Clinical responses in 18 patients include 2 PD, 8 CR and 8 PR (overall RR 89%). Both pts with PD had inflammatory cancer. Six out of 16 pts (37%) underwent breast conservation surgery. Pathologic CR occurred in 2/16 pts (12.5%). Three additional pts (18.7%) had evidence of pathologic CRm (only microscopic tumor present). Axillary LN dissection showed that 8/16 pts (50%) had no evidence of tumor, 6/16 pts (37%) had 1-3 LNs, and only 2 pts (12%) had 4-9 LNs involved. Perioperative CTC have been detected in 4 pts, interestingly, one of these pts was pathologically node negative with a residual 2-mm tumor. Toxicity including grade 4 mucositis, neutropenia, and hand-foot syndrome required dose reduction in 4 patients. Early recognition of hand-foot syndrome prompted a protocol change allowing 3 wks between D and T. Primary chemotherapy with dose-dense D and T given sequentially is both efficacious and tolerable in pts with LABC. We are encouraged by observed downstaging of nodal disease.

257 Epirubicin/Cyclophosphamide High Dose as Primary Chemotherapy in Locally Advanced Breast Cancer (BC)(T2-T4/N0-N2/M0) – Preliminary Data/Toxicities.

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Objective/Methods: Due to the emerging role of primary high dose Chemotherapy (CHT) we examined the rate of breast conserving therapy (BCT) in locally advanced BC. In 19 centers 143 patients (pts), 58 of them in the Womens Hospital Bayreuth - with BC primarily not suitable for BCT were treated with 3 cycles of Epirubicin (120mg/m2) and Cyclophosphamid (600mg/m2) in a prospective randomized trial. Pts. were randomized to a dose and time intensive arm A (d14 q3) or dose intensive arm B (d21 q3). To prevent serious neutropenic side-effects G-CFS was applied prophylactic depending on the blood count in arm B and from day 2-12 in arm A. 2-3 weeks after the 3rd cycle CHT surgery was performed. Pathologic evaluation of the tissue followed. In this paper data of the first 58 consecutive pts of the Womens Hospitel Bayreuth are evaluated in terms of tolerability, tumorreduction and quantity of BCT.

Results: In 8 cases treatment is in progress and not yet evaluable. Of the 50 evaluable pats. 2 pats. stopped treatment, one due to cardiotoxicity and one for personal reasons. After 3 cycles CHT BCT could be performed in 39 pts. (39/48, 81.2%); 9 of the pats underwent mastectomy (9/48; 18.8%). 27 of the pts showed pathological pos. nodal status. Pathological Tumour stage was: 19 times ypT1 (39.6%), 21 times ypT2 (43.7%), 6 times ypT3 (12.5%) and 2 times pathological complete response: ypTx (4.3%). According to the clinical results, the overall response rate (CR + PR) was calculated: RR=85.4%. Clinical complete remissions were found in 8.3 % of all tumours (4/48). In 144 cycles of chemotherapy the patients suffered from toxicities in 11 cases: neutropenia WHO gr. 3 in 3.5% (5/144), neutropenia WHO gr. 4 in 2.8% (4/144), neutropenic fever in 0.7% (1/144) and cardiotoxicity in 0.7% (1/144) of all cases. Besides most pts. suffered from alopecia WHO grade 3. Vomiting and diarhea were moderate. Follow up: Up to now there were two local recurrences, 3 new metastases and two BC related deaths in this treatment group. Data of all other participating centers will also be presented.

Conclusion: Downstaging of locally advanced BC is possible with primary EC-CHT, BCT was enabled in 77.5%. 66.2% of the tumours responded to this therapy with a reduction of the tumour size of more than 20%! EC-high dose CHT is a treatment with good feasibility and moderate toxicity in our experience.

258 Sequential Epirubicin (EPI)-Paclitaxel (PAC) Administration for Advanced Breast Cancer (BC). A Randomized Phase I Trial.

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A randomized phase I study was carried out to determine the maximal tolerated doses (MTD) without growth factors (GF) of a sequential administration of epirubicin and paclitaxel as first line treatment for advanced BC. The sequence A (EPI day 1 → PAC day 2) was compared to the sequence B (PAC day 1 → EPI, day 2). Patients ≥ 60 years or having received ≥ 300 mg/m^2 of adriamycine; ≥ 480 mg/m^2 of EPI or ≥ 60 mg/m^2 of mitoxantrone were excluded. Doses for the first cohort were 90 mg/m^2 for EPI and 175 mg/m^2 for PAC. Dose limiting toxicities (DLT) were classically defined. LVEF had to be closely monitored. 46 patients (median age: 47; 24 metastatic; 10 previous adjuvant anthracyclins) were included in the study. They received a median number of 5 (A) or 6 (B) courses (range 1-9). MTD was attained at level 1 for sequence B (2/6 DLT; 3/7 DLT in the confirmation cohort). In sequence A, MTD was defined at level 3 (EPI 100 mg/m^2, day 1; PAC 200 mg/m^2- Day 2); in this sequence, 3 DLT were observed among 33 patients treated (2/3 at level 4; 1/7 at level 1). DLTs were significant neutropenia in all 8 cases complicated by sepsis in 3 cases (2A – 1 B). Decrease in LVEF occuring in 10 cases (8/33 – A; 2/13 – B) was complicated in 4 cases (3A; 1B) by a congestive heart failure (CHF) with death in one case. CHF supervened after 7 or more courses; 3 patients had received previous adjuvant anthracyclin or mitoxantrone treatment and one had been irradiated on the left chest wall. Responses could be evaluated in 43 cases (10 CR and 25 PR (81 %) were recorded; 7 CR + 11 PR/20 (90 %) in locally advanced; 3 CR + 14 PR/23 (74 %) in metastatics. Previous adjuvant anthracyclins reduced the overall RR from 91 to 50 %. In loco-regional disease, residual intracanalar carcinoma was retrieved in 2 cases while pathological CR was assessed in only 3 cases. In conclusion, (1) the sequence A (EPI (EPI → PAC) was better tolerated than the reverse B; in the recommended doses for further phases II or III without GF are: EPI 100-day 1 and PAC 200 mg/m^2-day 2; (2) the high response rate observed, especially in loco-regional disease warrants further assessment.

259 The Correlation of Histone H3 Labeling Index and Post Chemotherapy PET Scan in Patients with LABC Treated with Neoadjuvant Doxorubicin and Docetaxel.

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Objective: To correlate histone H3 labeling index (LI) and postchemotherapy positron emission tomography (PET) in patients (pt) with locally advanced breast cancer (LABC) treated with neoadjuvant chemotherapy (CT). **Methods:** Pts with stage IIB (T3N0), IIIA, and IIIB LABC were prospectively treated with neoadjuvant CT of six cycles of Doxorubicin (50mg/m2) and Docetaxel (75mg/m2) every 21 days followed by modified radical mastectomy (MRM) and loco-regional XRT. PET scans using 18F-fluro-2-deoxy-D-glucose were obtained at diagnosis and at the end of chemotherapy. LI was determined on pre-treatment tumor biopsies, by in-situ hybridization using the histone H3 probe (DAKO) with standard techniques. LI was determined by counting 1000 cells. For data analysis LI of <7% was graded as low proliferation and >7% as high proliferation. Pathologic response was assessed as complete response (pCR) if there was no evidence of residual tumor in the resected specimen. Microscopic residual disease (pMRD) was defined as minimal areas of infiltrating carcinoma in not more than 2 high powered fields. Partial response (pPR) and stable disease (pSD) were defined as greater than 50% and less than 50% reduction in the volume as measured clinically prior to therapy, respectively. **Results:** 17 patients are available for analysis. Mean LI for this group was 7.04% with a range of 0-21.22%. 9 patients had a negative post CT PET scan. 7/9 (78%) had a low LI. Mean LI for this group was 5.61%. 1 pt with a cCR refused MRM. 5/9 (56%) pts had a pCR or pMRD. 3/9 (33%) had a pPR. 8 patients had a positive post CT PET scan. 2/7 (27%) had a low LI. Mean LI for this group was 8.9%. 8/8 (100%) patients had pPR or pSD. **Conclusions:** The correlation of histone H3 LI and post CT PET scan may be of value in assessing neoadjuvant CT response in patients with LABC. The small sample size is limiting. Further study and correlation with other prognostic factors is warranted.

260 A Phase II Neoadjuvant Trial of Sequential Doxorubicin and Docetaxel for the Treatment of Stage III Breast Cancer Measuring STAT Activation as a Predictor of Response to Therapy.

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Signal transducers and activators of transcription (STATs) are latent cytoplasmic transcription factors that are important in normal intracellular signaling. Over the past few years, evidence has been accumulating that points to a role for STAT family proteins in oncogenesis. To further assess the role of STAT signaling in breast cancer we developed a translational clinical research project to measure STAT activation in human breast cancers. Women with stage IIIa or IIIb breast cancer described as having at least a 5 cm palpable breast mass that was histologically confirmed as an invasive breast cancer are eligible to participate in this trial. Upon written informed consent women are required to undergo an incisional biopsy of both the primary breast mass and of normal breast tissue. Specific response markers (STAT activation, Bcl-2 family proteins, Her2/neu, SRC, EGFR, apoptotic index, and mitogenesis assays) are measured in both the tumor specimen and the normal tissue specimen. The neoadjuvant chemotherapy regimen is doxorubicin 80 mg/m^2 for three courses followed sequentially by docetaxel 100 mg/m^2 for three courses given intravenously every two weeks in a dose-dense fashion. Neupogen, 5 mcg/kg SQ from days 3 through 10 is utilized to maintain the dose-dense regimen. Our preliminary data on STAT activation in breast cancer reveals that approximately 80% of the tumors demonstrate elevated levels of STAT activity as compared to the normal breast tissue. By using antibodies to specific STAT family members, we have observed that STAT 1 and 3 are the primary subtypes that are activated in breast tumors. Sixteen patients have been enrolled to date and all of the patients that have completed therapy have had at least a partial response to the neoadjuvant regimen. Three complete pathologic responses have been observed. The regimen has been well tolerated. Dose reductions were required in 3 patients during docetaxel treatment due to grade III neuropathy and bone pain. The study is currently ongoing and a progress report will be presented at the conference.

261 Neoadjuvant High-Dose Sequential Chemotherapy with Adriamycin (A), Paclitaxel (P), and Cyclophosphamide (C) Is Feasible and Effective in Poor Prognostic Patients with Locally Advanced Breast Cancer (LABC).

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Background: Promising results have been reported for breast cancer pts with ≥ 4 positive nodes treated in adjuvant setting with 3 cycles of sequential APC (90/250 / 3000 mg/m^2 respectively) given at two-week intervals with G-CSF support and prophylactic antibiotic.

Patients and Methods: The same regimen was used as a neoadjuvant therapy for 14 pts with LABC with tumor ≥ 10 cm and/or extensive nodal involvement ≥ 2.5 cm in diameter.

Results: Pts median age was 41 years (range, 26 to 55). The median tumor size was 11 cm (range, 6 to 13); 7 and 7 pts had T3 and T4; 5 and 9 had N1 and N2; and N2 ≥ 2.5 cm in 10 pts. Total number of cycles given were: 118; A = 42; P = 38; C = 38. Complete and partial response was achieved in 11 and 3 pts, respectively for an overall response of 100%. All 14 pt had MRM that showed that 7 (50%) had no residual invasive carcinoma, 8 (57%) had no nodal involvement, and 5 (33%) achieved complete pathological response (negative breast and axillae). Grade III/IV toxicity included: neutropenia in 3% of cycles, febrile neutropenia in 3%, thrombocytopenia in 1%, and anemia in 2%. Dosages were reduced in 5% of the cycles, delayed in 7%, and omitted in 4%. Other grade III toxicity: hypersensitivity reaction in one pt, neurotoxicity/myalgia in eight, mucositis in seven, and bone pain in two. There was no toxic death, however, one pt developed fatal AML (M5, t 9/11) at 11 ms. At a median follow up of 12 ms, one pt died (AML), one pt is alive with disease (+18 m), and 12 alive with no evidence of disease.

Conclusions: Dose dense sequential chemotherapy is feasible and active in this poor prognosis sub group of pts with LABC.

262 P-Glycoprotein Expression Is Not Induced by the First Dose of Neoadjuvant Paclitaxel Treatment, So Probably Does Not Stop the Initial Apoptotic Response in Non-Responsive Breast Cancers.

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We investigated whether early apoptotic responses are related to breast cancer volume reduction, and whether early induction of P-glycoprotein occurs in breast cancers that do not respond to neoadjuvant paclitaxel chemotherapy. Eleven women with locally advanced breast cancer received paclitaxel (q 2 weeks, 4 cycles) as neoadjuvant treatment. Serial fine needle aspirations (FNA) (25-gauge, 1 pass) were obtained before treatment and at 24, 48, 72 and 96 hours after the first dose. Microscopic counts of apoptotic and mitotic indices were performed on H&E stained FNA slides. Two additional slides from each FNA (one fixed in formalin, the other in acetone) and formalin fixed tissue sections from the pre-treatment biopsy and the post-treatment resection were immunostained for P-glycoprotein. The change in cancer volume from treatment was determined using radiological measurements with allowance for change in the histopathologic amount of cancer. Apoptotic responses to the first paclitaxel dose were usually complete within four days. Non-responsive tumors had a truncated apoptotic response to paclitaxel. P-glycoprotein expression was observed around the cell membrane of positive control cells (multi-drug resistant MES-SA/MX2 cell line). P-glycoprotein expression was not identified in any of the serial FNA samples from any patient. Tissue samples before and after neoadjuvant paclitaxel treatment did not show cell membrane staining for P-glycoprotein. Although the duration and extent of apoptosis after the first dose of paclitaxel appeared to predict the amount of cancer reduction from this treatment, cessation of the initial apoptotic response in non-responsive tumors was not associated with induction of detectable P-glycoprotein expression.

263 Locally Advanced Primary Breast Cancer: Medium Term Results of a Randomised Trial of Multimodal Therapy Versus Initial Hormone Therapy.

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A prospective randomised trial was conducted to compare between multimodal therapy (neoadjuvant chemotherapy, Patey mastectomy, post-operative radiotherapy and adjuvant hormone therapy) and initial hormone therapy for locally advance primary breast cancer.

Fifty-six patients received multimodal therapy and 52 patients had initial hormone therapy. At a median follow-up of 52 months, there was no significant difference between the two groups, regardless of oestrogen receptor status, in terms of survival, rate of metastases, time to metastases and uncontrolled locoregional disease.

Subgroup analysis showed that, regardless of therapy mode, patients with oestrogen receptor positive tumours had longer survival, lower rate of metastases and better locoregional control when compared to those with oestrogen receptor negative tumours. Less than one third (31%) of patients who received initial hormone therapy have thus far required mastectomy. They also required less therapies to achieve disease control when compared with multimodal group (mean = 3.6 versus 4.9).

In conculsion, both approaches resulted in similar outcome in terms of local and systemic disease control. Advantage of the multimodal approach is a longer initial period of disease control (disease free interval) due almost entirely to locoregional control. Advantages of sequential approach with initial hormone therapy are reduced mastectomy rate and ultimate number of therapies required and hence the associated morbidity. It is therfore a resonable option for the management of locally advanced primary breast cancer, especially with oestrogen receptor positive tumours.

264 Gemzar, Adriamicyn and Taxol (GAT) in Primary Chemotherapy in Breast Cancer (BC): Preliminary Results.

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Our prior phase I-II study has demonstrated important activity with GAT combination in metastatic BC with 82,5% of response rate and confirm the feasibility of biweekly administration with a good toxicity profile. The use of neoadjuvant treatments is justified after randomized phase II trials (Fisher B et al. J. Clin Oncol 1997; 15: 2483-93, Makris et al, Ann Oncol 1999). Based on this considerations we started a pilot study with the aim to determine the efficacy in terms of objective and pathological response rate, rate of breast conserving therapy and role of moleculars markers in response of GAT combination in primary chemotherapy. Plan treatment was: six biweekly neoadjuvant cycles of G: 2000 mg/m2; A: 35 mg/m2; T: 150 mg/m2 with G-CSF support and another four cycles after surgery followed radiotherapy +/- hormonotherapy in 31 patients (p) with histologically confirmed invasive BC staged T2 (10p), T3 (7p), T4 (14p), N0-2. Median age: 58. PS: 0-1. Results: A total of 163 cycles were administered, 26 were delayed due to neutropenia or thrombocytopenia. Main toxicities was: neutropenia grade 3-4: 2% of cycles, neurotoxicity grade 1-2: 18% of cycles, myalgias grade 1-2: 23,3%of cycles. One patient had a transitory decrease of left ventricular ejection fraction (LVEF). The response rate was high (95,6% OR with 47% cCR). Pathologic findings are available in 22 patients; pCR was documented in 3 patients (16,3%) and 18,1% had microscopic foci < 1 mm. In terms of surgery 58,3% T2-T3 patients were benefited from conservative surgery. Conclusions: This combination shows a high response rate with an acceptable toxicity. The number of patients is still short to define the role of moleculars markers.

265 Hemoglobin Values during Neoadjuvant Chemotherapy Using Epirubicin, Paclitaxel Followed by CMF. Results from a Randomized Multicenter-Trial.

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During the last years preoperative chemotherapy for locally advanced breast cancer has been established. Especially if used in studies, the evaluation of new agents can increase the innovation in breast cancer treatment. The neoadjuvant ET-CMF trial conducted by the german AGO-group compares four cycles of Epirubicin(E)/ Paclitaxel(T) (90/175 mg/m2 q3w) with a sequential, dose-dense, -intensified regimen, using three cycles of E (150 mg/m2 q2w +GCSF) followed by three cycles of T (250 mg/m2 q2w +GCSF). Both preoperative treatment arms are followed by surgery and three cycles of CMF (500/40/600 mg/m2 d1/8 q4w). To evalute the changes in Hb values during treatment we analysed Hb before and weekly during the treatment. In 52 out of 330 randomized patients Hb values are available. At baseline patients in the sequential arm (A) had a mean Hb value of 13,8 g/dl (10,9-15,2) and patients in the standard arm (B) of 13,9 g/dl (12,7-15,8). Patients in arm A showed a mean decline of 3,2 g/dl, while in arm B it was 2,3 g/dl ($p<0,05$). Two patients received blood transfusions after the 3rd sequential cycle of Epirubicin. The mean of the minimum Hb was 10,6 g/dl (8,3-12,5)in arm A and 11,6 g/dl (8,5-13,3) in arm B ($p<0,05$). So far the results of this investigation show, that both treatment regimens lead to a significant drop of Hb, nevertheless this regimens both are practicable without the use of transfusions or Erythropoetin on a regular basis. We previously showed that therapy induced anemia in patients receiving dose-intensified chemotherapy is associated with a significantly higher risk of recurrence (Untch et al, Breast Cancer Res Treat Vol.57,NO.1,1999). Actual data indicate that low Hb values during treatment are not only combined with fatigue syndrom and reduced quality of life but may also be a predictor of weak response to therapy and poor outcome (Littlewood, ASCO 2000). Future trials will have to evaluate whether the correction of anemia during chemotherapy can improve the life quality and treatment outcome of breast cancer patients. Hb values of about 300 patients involved in the trial will be presented.

266 Combination Antiestrogen/Antiprogestin Therapy of MCF-7 Breast Cancer Cells Inhibits Cell Proliferation through an Rb-Dependent Pathway.

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Human MCF-7 breast cancer cells, which express functional estrogen and progestin receptors, were used to compare the efficacy of combined aniestrogen/antiprogestin treatment to either antihormonal therapy alone (monotherapy). The outcome parameter measured was inhibition of cell proliferation and the phosphorylation state of the tumor suppressor retinoblastoma (Rb) protein. Prior to experimental treatment, cells were estrogen depleted by culturing in pheno red-free DMEM Ham's F10 supplemented with decreasing concentrations of dextran-coated charcoal stripped serum. Subsequent to a 24 hour depletion of insulin to minimize the contribution of the insulin growth factor receptor pathway, cell monolayers were treated with 10 nM estradiol supplementation with and without tamoxifen (1.0 μM) and/or mifepristone (10.0 μM). A similar combination regimen with tamoxifen and mifepristone mediates optimal antitumor activitiy *in vivo* [Breast Cancer Res. And Treatment 49: 109-117, 1998]. Tamoxifen and mifepristone monotherapy similarly inhibit cell proliferation. Combined therapy further inhibits cell proliferation in an "additive" manner, as compared to monotherapy. Mifepristone and tamoxifen monotherapy inhibit Rb phosphorylation. Mifepristone-mediated inhibition of Rb phosphorylation is transient and occurs within the first 6 hours of treatment. In contrast, tamoxifen-induced inhibition of Rb phosphorylation is detectable only 12 hours after treatment, but is stably maintained throughout the treatment regimen. Thus, the additive increase in inhibition of proliferation by combined antiestrogen/ antiprogestin treatment occurs through an Rb-dependent pathway and involves a temporal differential of inhibition of Rb phosphorylation.

267 Molecular Mechanism of Action of a New Clinically Relevant Antiestrogen (GW7604) Related to Tamoxifen.

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New agents called selective ER modulators (SERMs) are being sought to improve the safety profile of tamoxifen so they can be used widely as preventives for breast cancer and osteoporosis. GW5638 is a tamoxifen derivative with a novel carboxylic acid side chain which preserves bone density but is an antiestrogen with no uterotropic activity in the rat.

We have compared the actions of 4 hydroxytamoxifen (4 OHT), the active metabolite of tamoxifen with GW7604 the presumed metabolite of GW5638 in breast (MCF-7) and endometrial (ECC-1) cell lines *in vitro*.

An assay was used that can classify SERMs into tamoxifen-like, raloxifene-like or pure antiestrogens depending on the activation of the transforming growth factor α (TGFα) gene *in situ* with wild type or asp351tyr mutant ER in stably transfected MDA-MB-231 cells. Computer assisted molecular modeling was used to observe the surface of antiestrogen ER complexes and to identify the critical interaction of the SERM side chains with individual amino acids.

The side chain of GW7604 changes the properties of the SERM from the promiscuous estrogen-like actions of tamoxifen that stimulates TGFα to the antiestrogen actions of a raloxifene-like compound. GW7604 and raloxifene stimulate TGFα with the 351 mutant ER. In contrast, ICI 182,780, a pure antiestrogen does not initiate TGFα. Using computer assisted molecular models of SERM ER complexes we have found that the side chain of 4-OHT weakly interacts with aa351 (carboxylic acid to tertiary amine) but the carboxylic acid of GW7604 causes a strong repulsion of aa aspartate 351. GW7604 did not cause the growth of ECC-1 cells at any concentration but 4-OHT was weakly estrogen-like at low concentrations. In conclusion, the compound GW7604 is active at the ER but is less estrogen-like in the SERM classification assay or ECC-1 cell line than 4-OHT. *Funded through the generosity of the Avon Breast Cancer Research Fund.*

268 Estrogen-Like Action of the Tamoxifen-Estrogen Receptor (ER) Complex: A Mechanism for Drug Resistance in Breast Cancer.

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Five years of adjuvant tamoxifen has been proven to produce survival advantages for women with node positive and node negative ER positive breast cancer. However, drug resistance to tamoxifen occurs. One form of drug resistance is tamoxifen stimulated tumor growth. Laboratory models of tamoxifen-stimulated tumor growth exist, but the molecular mechanism required for the conversion of the tamoxifen ER complex from an antiestrogenic to an estrogenic signal is unknown. Norris and coworkers (Norris JD,et al. *Science* 1999;285(5428):744-6) have proposed that coactivators bind at separate places on the tamoxifen ER complex and the estradiol ER complex to induce transcription. The discovery of a natural mutation in ER in a tamoxifen stimulated breast tumor now provides a valuable insight into a possible mechanism of drug resistance. The point mutation is the substitution of a tyrosine for an aspartate at aa351 (D351Y) (Wolf DM, Jordan VC *Breast Cancer Res Treat* 1994;31(1):129-38). Our goal was to investigate the potential activator binding sites on the surface of the ER through systematic mutagenesis. All mutant ERs were stably transfected into the ER negative breast cancer cell line MDA-MB-231 and gene activation of transforming growth factor α (TGFα) was our target *in situ* for bioassay. Our hypothesis was that the **inability** of the antiestrogenic side chain of tamoxifen to neutralize the aspartate acid on aa 351 results in estrogen-like actions. Raloxifene can neutralize the charge so it is complete antiestrogen in the assay. Since raloxifene becomes estrogenic with D351Y we reasoned the charge is not neutralized. Removing the charge at 351, to produce D351G, results in less of estrogen-like actions for tamoxifen but retention of antiestrogenic properties. However, removal of AF-1 (the A/B region) from ER removes estrogen-like properties for tamoxifen as does a triple mutation (D538A/E542A/D545A) in helix 12. We therefore propose a triple point attachment model for the binding of coactivators to negative charges on the tamoxifen ER complex. This site is distinct from the AF-2 binding site on the estradiol ER complex.

269 Effects of the Antiestrogens Tamoxifen and LY353381.HCl (Arzoxifene) on Endometrial Cancer Growth.

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Tamoxifen has been successfully evaluated as a breast cancer preventive in high-risk women and is the first choice of endocrine therapy for ER positive breast cancer patients. However, an important concern in the breast cancer patient on tamoxifen therapy is the 3-4 fold increased incidence of endometrial cancer. This fact has led researchers to search for compounds with estrogen like activity in bone and in serum lipids, but without activity in reproductive tissues. Arzoxifene is a novel benzothiophene analog with SERM activity similar to, but not identical with, raloxifene. Arzoxifene has been shown to have an antiestrogenic effect on the rodent uterus (Sato et. al. *J Pharmacol Exp Ther*, 1998; 287:1-7). Our objective was to evaluate the effects of tamoxifen and arzoxifene on the growth of human endometrial cancer in athymic mice to verify if there is cross-resistance for EnCa101 growth. Our study was conducted using a tamoxifen-naive and tamoxifen-stimulated human endometrial cancer (EnCa101) in ovariectomized athymic mice in response to estrogen capsules (0.3-cm), tamoxifen (0.5mg/d) and different doses of arzoxifene (0.5 and 1.5mg/d). The effects of tamoxifen and arzoxifene on the growth of either tamoxifen-stimulated or tamoxifen-naive endometrial tumors in athymic mice were not substantially different, independent of the dose. Arzoxifene and tamoxifen produce identical effects in our endometrial cancer models. Arzoxifene potentiates growth in the tamoxifen stimulated tumors and does not produce growth in tamoxifen-naive tumors compared to control group. Our data suggest that arzoxifene stimulates endometrial tumor growth in the setting of tamoxifen stimulated tumors in athymic mice to the same extent as tamoxifen, thereby predicting a limited role as a second line agent for the patient on tamoxifen who develops endometrial cancer.

270 Comparison of the Antiestrogenic and Estrogenic Activities of EM-652.HCl and Lasofoxifene in Human Endometrial Adenocarcinoma Ishikawa Cells and in the Ovariectomized Mouse Model.

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The mixed agonist and antagonist activity of the antiestrogen tamoxifen currently used in breast cancer therapy has stimulated the development of compounds having purer antiestrogenic activity in the mammary gland and endometrium. We have compared the antiestrogenic/estrogenic activities of EM-652.HCl with those of the selective estrogen receptor modulator (SERM) lasofoxifene (free base) on estrogen-sensitive alkaline phosphatase (AP) activity in human endometrial adenocarcinoma Ishikawa cells and on estrogen-sensitive organs in ovariectomized mice. The marked stimulatory effect on AP activity induced by 1 nM E_2 was competitively, but not completely, reversed by lasofoxifene at an IC_{50} value of 4.24 ± 0.86 nM while it was competitively and completely reversed by EM-652 at an IC_{50} value of 0.95 ± 0.10 nM. In addition, incubation of Ishikawa cells with increasing concentrations of lasofoxifene alone (in the absence of estrogen) caused a maximal 3.6-fold increase in AP activity, a half-maximal effect being achieved at 0.03 nM, while EM-652, at doses up to 1000 nM, had no stimulatory effect on basal AP activity. In mice, 9 day-treatment with EM-652.HCl at the daily oral doses of 1 µg, 3 µg, and 10 µg caused respective 24%, 48%, and 72% inhibitions of estrone-stimulated uterine weight ($p<0.01$ for all doses). Lasofoxifene had no inhibitory effect at the lowest dose used while it caused respective 25% ($p<0.01$) and 44% ($p<0.01$) inhibitions of estrone-stimulated uterine weight at the daily doses of 3 µg and 10 µg. Similar effects were observed on vaginal weight. When compounds were administered alone (in the absence of estrone) to ovariectomized mice at the daily oral dose of 10 µg, EM-652.HCl had no significant stimulatory effect on uterine and vaginal weights, while lasofoxifene caused respective 93% ($p<0.01$) and 73% ($p<0.01$) stimulations of these parameters. The present data show that the antiestrogenic activity of EM-652 is more potent than that of lasofoxifene in the models used and suggest an estrogenic activity of lasofoxifene on the parameters studied while EM-652 exerted pure antiestrogenic activity.

271 Antitumor Activity of Tamoxifen at Low Doses before Surgery.

Pigatto F, Veronesi P, Pelosi G, Bonanni B, Cazzaniga M, Guerrieri-Gonzaga A, Torrisi R, Arnone P, Bassi F, Robertson C, Decensi A. Chemoprevention Unit, European Institute of Oncology, Milan, Italy.

The use of tamoxifen as a chemopreventive agent is problematic because of the risk of venous thrombotic events and endometrial cancer observed in postmenopausal women. While the risk of endometrial cancer appears to be dose- and time-dependent, the therapeutic effect of tamoxifen at 20-40 mg/day does not follow a dose-response relationship. We have recently shown that 10 mg every other day of tamoxifen has a comparable activity than 20 mg/day on a set of biomarkers such as IGF-I and lipids. A dose reduction of tamoxifen is therefore a plausible means to decrease toxicity while retaining activity. We studied the activity of tamoxifen at low doses on a set of tumor tissue biomarkers. Women aged 45 years or older with ER positive stage I or II breast cancer following core biopsy were randomized to receive either 20 mg or 5 mg or 1 mg/day of tamoxifen in a double-blind 3-arm trial of 4 weeks before definitive surgery. In order to minimize differences in time to steady-state among the 3 doses, a single loading dose of 20 mg/day was administered in all subjects. The primary endpoint is the change in Ki67 between core biopsy and surgery. Secondary tissue endpoints are the change in ER and PgR expression and the change in morphometric parameters such as DNA index, nuclear area and chromatin texture.

As of April 30, 2000, 62 women aged 63±10 years have been randomized to tamoxifen and 18 women aged 63±8 years with negative ER tumours have been included as a control arm. Results on assessable subjects show a mean ±SD percent decrease in Ki67 of 31±41 from a baseline value of 25±13 in 52 subjects on tamoxifen and a mean ±SD percent increase of 1±25 from a baseline value of 44±22 in 13 untreated control subjects.

Our preliminary results suggest that Ki67 may serve as a useful surrogate biomarker to assess the activity of different doses of tamoxifen. Results by treatment arm will be presented at the conference.

272 Tamoxifen Effects on the Brain - Correlating Serial Positron Emission Tomography with Neurophysiologic Testing.

Mortimer JE, Donnelly J, Ball L, Knapp DL, Mintun M.

Clinical and experimental data support the role of estrogens in maintaining memory and cognitive function. The beneficial effects on the CNS have been attributed to a number of factors including increases in synaptogensis, acetyl choline synthesis and cerebral blood flow. Estrogen receptors have been identified in the brain, but are of uncertain functional significance. We have utilized positron emission tomography (PET) with the estrogen analog 16a-[18F]flouroestradiol-17β (FES) to identify in vivo hormone dependency in advanced ER+ breast cancer. The glucose analog [18F]fluorodeoxyglucose (FDG) is used to identify increased metabolic activity and cerebral blood flow. We sought to determine the effects of tamoxifen on memory and cognitive function correlating neurophysiologic testing with serial FES and FDG PET imaging before and after institution of the drug. Twelve women with breast cancer have undergone FES and FDG-PET at baseline and 6 weeks after institution of tamoxifen. Thusfar, FES-PET has identified areas of increased uptake in the limbic structures including the medial temporal lobe. We have yet to observe clinical changes in memory or cognitive functioning. However, the follow-up time is short, < 1 year. We continue to accrue patients for PET imaging and clinical followup data. Supported by a grant from the Charles A. Dana Foundation.

273 Differential Effects of Steroidal Type I and Non-Steroidal Type II Anti-Aromatase Agents.

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Drugs that specifically block the last step in estrogen biosynthesis (aromatase) are an attractive option for the treatment of hormone-sensitive breast cancer in postmenopausal women. Type I agents (e.g., exemestane, formestane) are steroidal and irreversibly inactivate the enzyme. Type II agents (e.g., aminoglutethimide, fadrozole, anastrozole, letrozole) are non-steroidal and reversible.

Two in vitro techniques have been used to determine the effect of anti-aromatase agents on estrogen biosynthesis in breast tumors and adipose tissue. In the first, in vitro aromatase activity was measured in excised tumor biopsy specimens obtained before and after systemic treatment with Type I (formestane) or Type II (aminoglutethimide) agents. Aminoglutethimide treatment produced a marked increase (2-75 fold) in aromatase activity in 12 of 13 patients, whereas formestane resulted in a decrease (>2 fold) in 9 of 10 patients. In a second study, cultured fibroblasts from mammary adipose tissue were preincubated with an anti-aromatase agent for 18 hours. Aromatase activity was then measured in the absence of drugs. Preincubation with fadrozole and anastrozole (1-100 nM) produced increased aromatase activity, whereas preincubation with the same concentrations of exemestane and formestane resulted in pronounced inhibition (>80%).

These results demonstrate that Type II reversible inhibitors have the potential to increase aromatase activity in vitro, whereas Type I irreversible inactivators consistently decrease activity. These effects illustrate the different mechanisms of action of the agents, which may have implications for clinical management.

274 Paradoxical Effects of Aromatase Inhibitors: Increased Activity of Target Enzyme Following Prior Exposure to Some But Not All Aromatase Inhibitors.

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Aromatase inhibitors block oestrogen biosynthesis and are increasingly used to treat postmenopausal women with breast cancer. The agents fall into two major types: steroidal inhibitors such as formestane and non steroidal inhibitors such as aminoglutethimide, fadrozole, letrozole and anastrozole. However we have reported that tumour material from breast cancer patients treated with aminoglutethimide displayed paradoxically increased levels of *in vitro* aromatase activity compared to levels in pre-treatment biopsies. To examine this phenomenon further, we have utilised a model system employing fibroblast cultures derived from breast adipose tissue.

Primary fibroblast cultures were obtained from mammary adipose tissue collected at mastectomy. To induce aromatase activity replicate cultures were incubated for 18 hours with dexamethasone (10μM) either in the absence or presence of varying concentrations of aromatase inhibitors. The results are as shown in the table.

The effects of aromatase inhibitors on aromatase activity. [values are % of control cultures without inhibitors and are medians of at least three separate experiments].

Concentrations	1nm	5nm	10nm	100nm	1μm	10μm
Fadrozole	124	112	140	128	155	
Anastrozole	133	135	132	125	133	
Aminoglutethimide				111	139	186
Letrozole	97	127	119	88	56	
Formestane	18	13	11	18	21	

Pre-treatment with fadrozole, anastrozole and aminoglutethimide all produced an increase in aromatase activity at each concentration tested. In contrast, whilst enhanced activity was evident with 5 and 10nM letrozole, higher doses were inhibitory. The effects of non-steroidal agents are to be contrasted with those of formestane which markedly inhibited activity at all concentrations employed. The contrasting effects between steroidal and non-steroid inhibitors probably relate to different mechanism of actions of drugs which could have implications in their clinical use.

275 Expression of a Novel Factor, com1, Is Regulated by Vitamin D in Breast Cancer Cells.

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Tumor cells and their surrounding microenvironment produce a variety of factors that promote tumor growth and metastasis. We recently identified a nuclear factor, termed com1, which is up-regulated in human breast carcinoma cells upon formation of experimental metastatic tumors and assumed to act as a growth-promoting factor in breast cancer. 1,25-Dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) is a potent inhibitor of growth in breast cancer both *in vitro* and *in vivo*. We compared the non-tumorigenic and estrogen-dependent MCF-7 cells with the tumorigenic and tamoxifen-resistant subline MCF7/LCC2 with respect to growth regulatory mechanisms in the presence of $1,25(OH)_2D_3$. Proliferation of MCF7/LCC2 cells, which revealed constitutive com1 expression, was inhibited by $1,25(OH)_2D_3$ (10^{-7} M). This was strongly associated with cell cycle arrest in G_1 phase, consistent with accumulation of the hypo-phosphorylated form of the retinoblastoma protein, as well as induction of the cyclin-dependent kinase inhibitor p21. These cell cycle events were preceded by a transient up-regulation (5- to 8-fold) of com1 mRNA. Furthermore, clonal growth of the MCF7/LCC2 cells was also inhibited by $1,25(OH)_2D_3$ (10^{-7} M), and when the com1-negative MCF-7 cells were stably transfected with com1, the resulting MCF7/com1 cells showed a significant decrease in colony formation. These results seem to indicate that rather than being growth-promoting, com1 may participate in the regulatory pathway involved in cellular growth inhibition when recruited by inhibitory signals.

276 OC Use and Breast Cancer - Effect on Tumorbiology and Prognosis.

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The question of whether oral contraceptive(OC) use before diagnosis has an effect on tumor biology and prognosis of breast cancer remains a subject of discussion.

A nested case-control study was conducted to investigate the effect of OCs on prognostic factors and the long term survival of breast cancer patients. Results of a 10 year median follow-up will be presented. In 471 breast cancer patients histomorphological (tumor type, grading, tumor size, nodal status, ER, PR) and molecularbuiological factors (PCNA, EGF-R, c-erbB2, p53) and their association to OC use were studied.

In current users a significant increase in node-positive tumors (OR 2.14),poorly differentiated tumors (OR 2.01)and in highly proliferating tumors (OR 2.69) was observed. Past users had significantly more ER-positive tumors compared to never users (OR 2.16-2.69)combined with an increased EGF-R expression. Multivariate survival analyses showed a significant improvement in survival for long-term users (HR 0.55, 95%CI 0.34-0.90) and first use more than 8 years before diagnosis (HR 0.49, 95%CI 0.34-0.90), whereas current use was related to a significant decrease in survival rates (HR 2.29, 95%CI 1.02-5.17). Thus OC use covering a biologically sensitive period in tumor development might be more important for the prognosis of breast cancer patients than duration of use. A biological hypothesis will be suggested.

277 Estrogen Receptor Beta Is Expressed by Human Breast Fibroblasts.

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Two isoforms of the estrogen receptor (ER) are currently known ER alpha (ERα) and ER beta (ERβ). ERβ, a recently described isoform, represents a separate gene product with distinct biological roles and ligand binding specificity when compared to ERα. There is limited data on the protein expression of ERβ within the human breast and the possible presence of ERβ within the human breast stroma has not been reported. We have done western blotting on 24 breast samples (5 benign and 19 malignant) and found ERβ in all samples. RT-PCR of 8 samples revealed that a c-terminal truncated region (βcx) variant (known dominant negative receptor) was present in all these samples. Immunohistochemistry using a novel ERβ antibody (503) was done on 6 breast samples (1 normal and 5 malignant) and this revealed that 5/6 showed ERβ positive nuclear staining of epithelial cells, in addition staining of breast fibroblasts was seen. Using a cell purification system developed within the laboratory normal breast fibroblasts were obtained from healthy women following mammoplasty. Using this purified population the presence of stromal ERα and ERβ was investigated in fibroblasts by RT-PCR and slot blot. ERβ was detected by both methods in human breast fibroblasts. RT-PCR revealed the presence of wild type (wt) and βcx variant of ERβ, but not of the exon 5 deletion type (δ5). Each sample had variation in relative amount of wt and βcx. We could find no evidence to date of the apparent presence of stromal ERα. The role of fibroblast growth factor 7 (FGF7), a known paracrine mediator of mesenchymal-epithelial interaction, to act as an estromedin within the breast was investigated by treating normal human breast fibroblasts with estradiol and measuring released FGF7 by using an FGF7 ELISA. In contrast to interleukin-1beta (IL-1β) which shown a marked release of FGF-7, estradiol had no effect. This raises the possibility that there may be a further ligand for breast stromal ERβ.

278 Association of Breast Cancer and Estrogen Receptor Gene Polymorphism.

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The determination of estrogen receptor positivity by immunohistochemistry is a well accepted and widely used predictive factor for response to anti-hormonal treatment in breast cancer. The phenotypic expression of the estrogen receptor could be influenced by a genetic polymorphism of the estrogen receptor gene. In this epidemiologic study, we investigated 1. the distribution of the estrogen receptor polymorphism PvuII (P/p) in breast cancer patients versus aged-matched controls and 2. the correlation of the PvuII genotype distribution with the phenotypic estrogen receptor status as evaluated by immunohistochemistry.

We extracted DNA of peripheral white blood cells from 236 breast cancer patients and an equal number of healthy control persons. DNA was amplified by polymerase chain reaction (PCR). The PCR product was digested by the restriction enzyme PvuII at the corresponding restriction fragment length polymorphism (base exchange T→C in intron 1 of the estrogen receptor).

There was no statistically significant difference in the estrogen receptor genotype distribution between breast cancer patients and controls (Table 1). However, the genotype PP showed a statistically significant overrepresentation as compared to pp + Pp in breast cancer patients versus controls (odds ratio: 1.57; confidence interval: 1.021-2.415, p=0.039). An association between the estrogen receptor status and the immunohistochemical phenotype of the estrogen receptor could not be found.

In this analysis, we could show a increased risk to develop breast cancer for the PP genotype of the estrogen receptor polymorphism which could be integrated in molecular biological profiling and screening programs of high-risk families. The estrogen receptor status by immunohistochemistry is not associated with the base exchange in intron 1 of the estrogen receptor.

Genotype	PP	Pp	pp
Breast Cancer (n=236)	27.5%	49.2%	23.3%
Controls (n=236)	19.5%	55.1%	25.4%

279 Identification of a Novel Variant Estrogen Receptor Transcript in Human Breast Cancer.

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Using [^{3}H]tamoxifen aziridine labeling agent, we previously detected in undifferentiated tumors and malignant lymph nodes a 43 kDa truncated estrogen receptor (ER) unable to bind estradiol (Breast Cancer Res Treat 1996; 40:231-241). This peptide was recognized by a panel of anti-ER antibodies directed against amino acid sequences located at both N- and C-terminal domains of the wild-type 67 kDa ER. Remarkably, 3 antibodies against segments encoded by exons 4-6 were ineffective suggesting a truncation in the central region of ER.

ER cDNAs from 15 primary breast cancer samples were amplified by PCR using primers situated in exons 3 and 7. Three fragments were clearly seen after electrophoresis. Southern hybridization of RT-PCR products with an ER cDNA probe (1300 pb, EcoRI fragment of pOR3) revealed 3 fragments, i.e. wild-type 765 pb, exon 4 deleted 429 pb and unidentified ~230 pb (validity of methodology established with a panel of control ER-positive and ER-negative breast cancer cell lines). Differential hybridization of the membrane was performed with four probes in exons 3, 4, 5 and 6 respectively. Probe for the 3' end of exon 3 hybridized with all fragments; all other probes recognized solely the wild-type cDNA indicating partial or total deletion of exons 4, 5 and 6 in the ~230 pb fragment. Northern blot analysis of ER mRNA detected only the wild-type transcript probably due to low expression of the variants. These data support our view that transcript corresponding to ~230 pb fragment may encode for the 43 kDa ER peptide. Cloning and sequencing of this fragment are under investigation to further assess this hypothesis.

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280 Feasibility of Measuring Gene Expression Patterns Using Core Biopsies of Human Primary Breast Cancers and cDNA Microarray Technology.

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Introduction Identification of predictive markers for chemosensitivity would make it possible to individualize systemic therapy for patients with early breast cancer. With the advent of high-throughput quantitation of gene expression and cDNA technology, it is now possible to study the expression of many genes simultaneously. The main purpose of this study is to examine the feasibility of measuring gene expression using core biopsies of primary human breast cancers. Methods Tissues from human primary breast cancers were obtained by core biopsy using a 14-gauge Bard MaxCore Biopsy Instrument in 2 women presenting with primary breast cancers > 4 cm. These core biopsies were longitudinally cut into 8mM sections, mounted onto microscope slides, and rapidly air-dried for RNA extraction. RNA was also obtained from a 10 mM section of an excisional biopsy of a primary breast cancer from our frozen tumor bank. The RNA from each sample was reverse transcribed into ^{32}P-labeled cDNA using a primer kit (Clontech AtlasTM Human Trial kit, Palo Alto, CA) specific for an array of 96-genes and control DNAs spotted in duplicate on nylon. After hybridization and washing, the expression profiles from the core biopsies were compared to each other and to RNA obtained from excisional biopsy. Results The H&E stained histological sections showed approximately equal tumor cellularity in all the three specimens (> 70% tumor cellularity). Each core biopsy measured about 2 cm by 1 mm. Approximately 2 mg of total RNA was isolated from each biopsy (~1mg/mg wet tissue weight). The 91 genes on the array showed differences between the three tumors. The core biopsies overexpressed SOD1, CDK2, and showed increased DCC expression relative to the excisional tumor. The excisional tumor failed to express RB and overexpressed C-MYC and INS, relative to the core biopsies. The results from these preliminary studies indicate it is possible to detect characteristic RNA expression profiles for individual tumors from core and excisional biopsies of human breast cancers. Conclusion We have determined it is feasible to detect characteristic RNA expression profiles for individual human breast cancers using cDNA microarray technology. We have now initiated a study to examine whether cDNA technology could detect differential gene expression patterns between responders and non-responders as a predictive test for chemotherapy sensitivity.

301 Can Her-2/neu Select Patients to Primary CMF?

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Introduction: Her-2/neu is amplified/overexpressed in 25-30% of breast carcinomas. It has been related to poor outcome in patients treated with CMF.

Aims: To evaluate in primary CMF whether HER-2/neu status: 1. Predicts the response; 2. Influences the disease free and overall survival.

Material and methods: From 7/89 to 12/99, 304 primary breast carcinomas greater than 29 mm were treated with three cycles of neoadjuvant CMF. HER-2/neu overexpression was determined by immunohistochemestry (IHQ) with two antibodies (monoclonal CB-11 from Biogenex and polyclonal anti-HER-2/neu from Dako). Fluorescence in situ hybridisation (FISH) with locus her-2/neu specific probes (Oncor-Ventana) was used to detect amplification in the cases with discordant IHQ results. The response to chemotherapy was assessed before surgery by mammography. Response was defined as a > 50% shrinkage of the two largest tumour diameters.

Results: HER-2/neu overexpression was found in 17% (monoclonal Ab) and 37% (polyclonal Ab) of the cases. Overall HER-2/neu amplification/overexpression combining both IHQ and FISH results was 24%. C-erbB-2 was positive in: 9% nuclear grade 1 assessed by cytology, 25% grade 2 and 26% grade 3 tumours (p=0.2); 30% ER negative and 20% ER positive tumours (p=0.05); 27% PR negative and 21% PR positive tumours (p=0.3). No differences were seen in the mean number of positive lymph nodes after surgery (p=0.6).

No significant statistical differences were observed in the response rate between HER-2/neu positive and negative tumours: 51.3% vs. 47.3% (p=0.55). Moreover, five years disease free survival (65% vs 63%) and overall survival (82% vs 78%) showed no statistical differences.

Conclusions: In our series of breast carcinomas, Her-2/neu amplification/ overexpression does not predict the response to primary CMF. Patients outcome is not influenced by Her-2/neu status.

302 Clinical-Pathologic Assessment of the Clinical Sensitivity to Single Agent Taxane (T) Therapy for Metastatic Breast Cancer (MBC).

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The taxanes (T), paclitaxel and docetaxel, cause mitotic arrest with accumulation of cells at the G_2-M interphase. T have been reported to induce apoptosis via a non-p53-mediated pathway. A checkpoint blockade at the G_1/S boundary would be expected to promote T-induced apoptotic cell death, via a mechanism that may involve p27. Lower p27 levels in breast cancers assessed by IHC have been associated with shorter survival (Porter et al. Nature Med 3:222-5, 1997). Furthermore, increased p27 expression has been observed in paclitaxel-resistant 435/TO.3 breast cancer cells compared to its parental MDA 435 cell line (St. Croix et al. Nature Med 2:1204-1210, 1996). Other proposed predictors of clinical T sensitivity/resistance include p53, epidermal growth factor receptor (EGFR), HER2/neu, as well as estrogen and progesterone receptors (ER and PR). We performed IHC for ER, PR, EGFR, HER2/neu, bax, bcl-2, p53, and p27 on 144 breast tumor specimens from patients treated for MBC on a series of phase II clinical trials of single agent T for correlation with clinical response (CR/PR). Patient demographic characteristics that could influence response, i.e. performance status, extent of disease, extent of prior therapy, visceral dominant disease, and prior anthracycline exposure were also examined. In univariate analysis Karnofsky Performance Status (KPS) ≥ 90 and no prior history of anthracycline therapy correlated with a good clinical response to T (p=0.003 and 0.041 respectively). None of the IHC variables tested were predictive of clinical response to T, although p27 negativity showed a trend towards significance (p=0.075). This trend was maintained in multivariate analysis when adjusting for "extent of metastatic disease" and "prior doxorubicin". Neither ER nor PR were predictive of clinical response to T therapy (p=0.931 and 0.995). No association was observed between response to T chemotherapy and Her2/neu status, as shown by either HercepTest (DAKO) or CB-11 (Ventana). The definition of molecular phenotypes as predictive factors for taxane benefit remains an important goal. Support DAMD17-94-J-4329

303 Evaluation of Predictive Factors in a Randomized Trial of Preoperative Dose-Intensified Adriamycin-Docetaxel +/- Tamoxifen in Primary Operable Breast Cancer.

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Evaluation of predicitive factors for the efficacy of systemic treatment in primary breast cancer is best possible in the setting of preoperative chemotherapy. We have studied conventional prognostic factors (menopausal status, tumor size and nodal status by palpation, grading) and immunohistochemically detected biological factors (estrogen (ER)- and progesterone (PR) receptor content, Ki-67, Her2/neu, p53, and bcl-2) in 250 patients with T>3cm, N0-2, M0 breast cancer. All patients have been treated in the GEPARDO-trial (Proc ASCO 2000 # 322) with a dose-intensified combination of adriamycin (50 mg/m²) and docetaxel (75 mg/m²) before breast surgery. 122 patients have been prospectively randomized to simulateous tamoxifen treatment. Logistic regression analysis for detection of pathologic complete response (pCR) was performed.

Node negative, undifferentiated, ER- and PR-negative and bcl-2 negative tumors showed a significant higher pCR-rate in univariate analysis. In multivariate analysis nodal status, grading, and Her2/neu were significant predictors of pCR with odds ratios between 5 and 6.4 (Table). In a backward selection procedure nodal status and ER-status remained as the only significant predictors.

Clinically node negative patients with ER-negative tumors have the highest probability to experience a pCR using a dose-intensified combination of adriamcyin and docetaxel +/- tamoxifen as preoperative chemotherapy in primary operable breast cancer.

factor	groups	Wald-test p	odds ratio
menopaus	pre /peri+post	0.3	1.9
tumor size	<4 / > 4 cm	0.8	0.8
grading	3 / 2-1	0.04	5.0
nodal status	neg / pos	0.01	6.4
tamoxifen	no / yes	0.4	1.6
er	0-9 / 10-100%	0.3	3.0
pr	0-9 / 10-100%	0.3	3.9
her/2 neu	0-2+ / 3+	0.05	5.8
ki-67	0-15 / 16-30/31-100%	0.6	0.4
p53	1-50% / 0+51-100%	0.3	0.7
bcl-2	0-1+ /2-3+	0.1	5.7

304 Can Changes in Proliferation Predict Response to Tamoxifen in Breast Cancer?

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This study investigates the effects of neoadjuvant tamoxifen on proliferation of breast cancer using MIB1 antibody with immunohistochemistry and correlating changes during and after treatment with response.

50 postmenopausal women with large (>3cm) ER rich breast cancers were treated with tamoxifen 20mg a day for 3 months. Biopsies were taken at diagnosis, 10-14 days after starting treatment and at 3months. Tumours were monitored by clinical examination, ultrasound and mammography and response defined as ≥25% reduction in tumour volume. Quantitative immunohistochemistry using MIB1 antibody was performed using image analysis; the percentage of cells staining positive was assessed. 38 of 50 patients (76%) responded to tamoxifen.

MIB1 staining was significantly reduced at 10-14 days and 3 months in responding tumours but not non-responders. At 10-14 days 28/38 (74%) of responding tumours compared with 3/12 (25%) non-responders showed a decrease in staining, a significant difference between groups, p=0.005.

In the majority of responding tumours changes in proliferation can be detected which predate changes in tumour volume. By combining MIB1 with other markers such as apoptotic indices it may be possible to more precisely define shortly after starting treatment those who will respond.

305 N-Terminally Truncated HER-2 Protein, p95, Is Associated with Lymph Node Metastasis.

Ramsey BE, Keenan EJ, Sexton G, Clinton GM.

The HER-2 oncogene has limited prognostic value in node negative breast cancer. We previously reported a truncated kinase domain p95HER-2 in breast cancer tissue,which is the cellular remnant of shedding of the extracellular domain of p185HER-2. P95HER-2, but not the full length p185HER-2,was associated with factors that predict poor outcome. The goal of the current study was to use a second,independent patient population to examine the association of p95 with negative risk factors and to analyze the levels of p95 in metastatic breast cancer tissue. Tumor tissue was extracted, fractionated, subjected to Western blot analysis, and scored for levels of p95HER-2 and p185HER-2. A higher proportion of tissue from node positive patients (11 of 45) than node negative patients (6 of 49) was positive for p95 (p=.0495). Moreover, metastatic lumph nodes contained a higher level of p95HER-2(20 of 81)than tissue from local recurrence (4 of 37; p=.040). In contrast, p185HER-2 was not significantly associated with lymph node metastasis. When both patient populations were combined, 34 of 95 samples from node positive patients were positive for p95 while 15 of 91 samples from node negative patients were positive for p93 (p=.005). Our results provide additional evidence that p95 HER-2 is associated with lymph node metastasis and that it may be of value in predicting outcome in node negative breast cancer patients.

306 Choice of Chromogen Can Affect Determination of HER2 Status by Immunohistochemistry.

Dressler LG, Cowan D, Geradts J, Wang WY, Moore D, Little D, Miller A, Vick C, Newman B, Millikan R. Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, NC.

The optimum method to determine HER2 status is uncertain. We have previously reported the comparison of two methods to determine HER2 status in 527 invasive breast cancer cases: immunohistochemistry (IHC) using CB11 antibody with DAB (3',3' diaminobenzidene) as chromogen and amplification using differential PCR (D-PCR). Nearly 20% of cases showed discordant results: 12% had overexpression (OE) without amplification (AMP) and 7% had AMP without OE. Weak staining cases by DAB-IHC were frequently negative by PCR(Dressler et al, AACR, 2000). In the present study, we performed IHC using the same primary antibody (CB11, Biogenex) but a different chromogen, Vector SG (dark gray color) to visualize HER2 membrane staining in a subset of the 527 cases. Using SG chromogen resulted in better quality immunostaining: crisp, distinct membrane staining with minimal cytoplasmic staining was observed compared to results using DAB. Using the same definition for IHC positive (OE), we compared IHC results using DAB and SG chromogens. We also compared IHC results using each chromogen with amplification results by D-PCR. All sections used for SG-IHC assays were cut from the same block used for DAB-IHC and PCR assays and were confirmed to contain representative invasive breast cancer.

Of the 278 cases randomly selected for this study, 24% (68/278) were amplified by D-PCR, 32% (89/278) were OE by IHC using DAB and 16% were OE by IHC using SG. Comparing results between SG and DAB IHC, 51 cases were assigned a different HER2 status: 48 cases (most showing a weak membrane stain)were positive by DAB, but negative by SG; 43 of these cases were also negative by PCR. Three cases were negative by DAB (cytoplasmic staining only) but positive by SG: one of these three cases had strong membrane staining. PCR showed amplification only in the case with strong membrane staining by SG. Using D-PCR as a reference, overall concordance for assignment of HER2 status (positive vs. negative) was higher with SG (82%) compared to DAB (69%); specificity was markedly improved using SG (94%) versus DAB (75%); sensitivity was slightly decreased using SG (46%) versus DAB (51%). Conclusion: Although SG chromogen may improve specificity of the IHC assay compared to DAB, weak membrane staining by IHC should be confirmed using amplification methods.

307 Lack of Interaction between Tumor Histologic Grade and Response to Chemotherapy in Node Negative and ER Negative Breast Cancer.

Park K, Yothers G, Bryant J, Wolmark N, Paik S. Pathology, Biostatistics, and Operations Center, NSABP.

Background: NSABP B-13 and other trials have demonstrated the clinical benefit from adjuvant chemotherapy for node negative ER negative patients. Previous studies have demonstrated a greater treatment effect in poorly differentiated tumors (Fisher et al, Cancer 15:181-191; Neville et al, JCO, 10:696-705). In order to test the hypothesis of a treatment-histologic grade interaction in node-negative and ER negative breast cancer, we conducted a retrospective analyses of cases enrolled in B-13.

Method: H&E stained slides from 645 of 731 patients who participated in NSABP B-13 and randomized to surgery with/without chemotherapy (MF) were available for review. Nottingham Histologic Grade (NHG) was used. Statistical tests to evaluate the interaction between treatment effect and NHG were performed.

Results: With median follow-up of 13.2 years, improved DFS and OS outcomes were seen in all NHG subgroups including NHG 1 (summarized in the table). There was no evidence for treatment-NHG interaction.

	NHG	RR of chemotherapy	Treatment p	Interaction p
DFS	1 (11.0%)	0.59 (0.26-1.35)	0.0001	0.54
	2 (39.8%)	0.68 (0.47-1.00)		
	3 (49.2%)	0.55 (0.39-0.79)		
OS	1	0.29 (0.09-0.99)	0.026	0.59
	2	0.88 (0.55-1.41)		
	3	0.62 (0.40-0.96)		

Conclusion: For node negative and ER negative patients, NHG failed to identify a subset of patients who did not benefit from MF chemotherapy.

308 Self Organizing Maps and Prognosis of Advanced Breast Cancer Patients with Bone Metastases Receiving Letrozole or MA.

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INTRODUCTION: The aromatase inhibitor Letrozole 2.5mg has previously shown superiority over megestrol acetate (MA) as second-line therapy in postmenopausal women with advanced breast cancer. The purpose of this paper is to analyze the efficacy of Letrozole or MA with respect to objective response (OR), clinical benefit (CB = CR + PR + SD>6 mths), time to progression (TTP) and overall survival (TTD) for pts with bone metastases and to build a model predicting the outcome using self organizing maps (SOMs).

METHODS: 171 ABC-patients with bone metastases [Mean age: 64.5] were randomly assigned to receive letrozole 2.5 mg (L25; n =52), letrozole 0.5 mg (L05; n = 59), or MA (n = 60) in a double-blind, multinational, peer reviewed trial. Data were analyzed using descriptive statistics, logistic and Cox regression, neural nets (SOMs) and inductive learning algorithms (C4.5).

RESULTS: There is no significant difference in response rate between L25, L05, and MA (OR:p =.70; CB: p=.78). Although there is a trend in favour of L25 there is no significant difference in duration of response between L25, L05, and MA (log rank p=.28). The most important parameter for CB is performance status (p=.03) (e.g. 35.8% of pts with grade 0 have CB; if in addition body mass < 30kg/m2 then CB is 40% for pts receiving L25 or L05).

*	CR	PR	SD	PD	NA/NE	OR	CB	TTP(OR)	TTP(CB)	TTD(CB)
L25	1.9%	17.3%	7.7%	65.4%	7.7%	19.2%	25.0%	819	819	1089
L05	0%	13.6%	18.6%	44.1%	23.7%	13.6%	30.5%	851	555	1083
MA	0%	15.0%	16.7	56.7%	11.7%	15.0%	30.0%	505	440	933

* TTP(X) = median duration of X (days); TTD (CB)= median survival time of pts with CB (days).

The SOM-model, built from data of the trial (n=171), was used to predict the outcome of pts in a different trial (n=68). The overall error for OR is < 10%. There were no significant differences between calculated TTP[e.g. L25: mean (median) TTP: 245.3d (115.1d); 95% CI: 168.1; 322.4 (56.3;173.8)] and actual TTP [mean (median)TTP:256.2d (119.0d); 95% CI: 192.5;319.8 (57.7; 180.2)].

CONCLUSION: In contrast to visceral and soft tissue metastases, there are no significant differences in outcome for ABC-pts with bone metastases with respect to treatment with letrozole or MA. SOMs can be used to predict the clinical outcome for an individual patient with a level of confidence of 90% and more.

309 Amplification of Topoisomerase IIa or c-erbB-2 Predicts Response to Doxorubicin and Docetaxel in Locally Advanced Breast Cancer.

Coon JC,[1] Marcus E,[2] Gupta-Burt S,[1] Seelig S,[3] Jacobson K,[3] Fronda G,[2] Preisler H.[1] [1]Rush University, Chicago, IL; [2]Cook County Hospital, Chicago, IL; [3]Vysis, Inc., Downers Grove, IL.

The overall goal is to identify molecular targets of tumor cells which predict pathologic response (PathR) to specific chemotherapy (CTX) at mastectomy in locally advanced breast cancer (LABC). Patients with stage IIb (T3N0), IIIA, and IIIB LABC were enrolled in a prospective CTX trial evaluating 6 cycles of Doxorubicin (50mg/m2) and Docetaxel (75mg/m2) followed by mastectomy and loco-regional radiation. Copy number of topoisomerase IIa (topo2), c-erbB-2, and chromosome 17 were determined by multicolor fluorescent in situ hybridization (FISH, Vysis probes) in pretherapy core biopsies from 20 patients. Expression of c-erbB-2 (Dako polyclonal antibody) and topo2 (Neomarkers clone JH2.7) were measured by immunohistochemistry and scored 0 to 4+. All 3 patients with topo2 amplification (amp) had a complete response (CR) or minimal residual disease (MRD) at mastectomy, versus 3 of 17 (18%) without amp (p=0.003). The other 14 patients had a partial response, stable disease, or progressed. Four of 5 patients with c-erbB-2 amp had CR or MRD versus 2 of 15 without amp (p=0.005). All tumors with topo2 amp had c-erbB-2 amp. High expression of topo2 was also associated with CR or MRD (4 of 6 patients had CR or MRD, p=0.03), but overexpression of C-erbB-2 was not (4 of 10 patients had CR or MRD, p=0.10). Conclusion: Amplification of topo2 or c-erbB-2 by FISH predicts excellent response to this treatment in LABC. More cases are needed to determine the stronger marker, but other data point to topo2 for anthracyclines.

310 Response Assessment by HER2/neu Status in a Trial with Dose-Intensified Weekly Paclitaxel in Metastatic Breast Cancer.

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Due to preclinical data and the results of the phase III trial with chemotherapy plus Herceptin versus chemotherapy alone, the sensitivity of HER2/neu positive breast cancers to paclitaxel was often questioned [Slamon et al., 1998]. Circulating levels of the shed, extracellular domain of HER2/neu have been shown to be a predictive parameter for drug response to cytotoxic and anti-hormonal therapy of HER2/neu positive patients. We treated 44 patients with metastatic breast cancer with dose-intense fractionated paclitaxel as 2nd- or 3rd-line chemotherapy (90 mg/m2 weekly x6, q9w) and correlated the staging results (UICC criteria) with the baseline levels of the circulating HER2/neu antigen. In addition, the HER2/neu serum results at stage IV disease were compared with HER2/neu immunohistochemistry (IHC) by the DAKO HercepTest® and fluorescence in situ hybridisation (FISH) status (Ventana® probe) of the primary tumor.

Using a cut-off of HER2/neu positivity of 15 ng/ml for the Oncogene Science® (Cambridge, MA, USA) kit, we found that 68% of patients had elevated levels of the ECD of HER2/neu which is in line with HER2/neu serum results for stage IV disease of other investigators [Andersen et al., 1995]. In 3/44 cases, HER2/neu results of the primary tumor were discordant for IHC (+) and FISH (-). These 3 patients were negative for serum HER2/neu in the metastatic setting. The overall response rate (RR) for all patients to weekly paclitaxel therapy was 39%. While the RR among HER2/neu positive patients was 47%, HER2/neu negative patients responded in 22% only. Although this difference was statistically not significant (p=0.65), most probably for the moderate patient number in this phase II trial, the data indicate that HER2/neu positive patients are equally sensitive to dose-dense paclitaxel as compared to HER2/neu negative patients.

Our results suggest that a negative prognostic and predictive factor like HER2/neu positivity may be overcome by (dose-dense) systemic therapy. The HER2/neu serum level is a very convenient method to assess the current HER2/neu status at multiple time points of therapeutic decisions and must be considered a complementary method to IHC and FISH of the primary tumor.

311 Immunomarkers on Cell-Blocks from Fine-Needle Cytopuncture as Predictors of Tumor Response to Preoperative Chemotherapy in Non-Metastatic Primary Breast Carcinoma.

Briffod M, Cohen-Solal C, Hacène K, Le Doussal V, Tubiana-Hulin M. Centre René Huguenin de Lutte contre le Cancer, Saint-Cloud, France.

We evaluated the predictive value of biological markers by immunohistochemistry on cell blocks from diagnostic fine-needle cytopunctures for tumor response to preoperative chemotherapy in a group of 105 breast carcinoma treated from 02/96 to 02/2000.

Patients had non-metastatic large primary breast carcinoma (T2>30mm-T3-T4, N0-N2), non-inflammatory (n=98) or inflammatory (n=7) and received anthracycline-based regimen, (FEC 60 to 100 (n=30), AC (n=52), AT(n=23), before surgery. Immunohistochemistry for MIB1 (Ki67), estrogen receptor (ER), progesterone receptor (PgR), p53 and c-erbB-2 was performed in all cases.

Nine patients achieved pathologic complete regression (pCR), 43 were scored partial regression (PR: concordant clinical, mammographic and histologic findings) and 53 were scored no regression (NR). Objective tumor regression (pCR+PR) was related to Ki67 ($p<0.0005$), ER ($p<0.02$), p53 ($p<0.05$) and c-erbB-2 ($p<0.01$). All tumors that showed pCR had high Ki67 (≥60% of positive nuclei), low or medium ER and PgR (<50% of positive nuclei) and overexpression of c-erbB-2 (≥10% of positive cells); none of them were inflammatory. Inversely, only 9/53 NR had a similar immunohistochemical profile.

Conclusion: Our results are in agreement with others that have shown that markers of proliferation (Ki67 and c-erbB-2) and hormonal receptors are useful to predict tumor regression to anthracycline-based neoadjuvant chemotherapy.

312 Immunohistochemical Variation of Human Equilibrative Nucleoside Transporter 1 Protein in Human Primary Breast Cancers.

Mackey JR,[1,2] Jennings L,[2] Dabbagh L,[3] Vsianska M,[5] Koski S,[1] Young JD,[3] Coupland R,[2,4] Cass CE.[2] [1]Division of Medical Oncology; [2]Division of Experimental Oncology; [3]University of Alberta; [4]Department of Pathology, Cross Cancer Institute; [5]Masaryk Memorial Cancer Institute, Brno, Czech Republic.

Physiologic and therapeutic nucleosides are generally hydrophilic, and require plasma membrane nucleoside transporters for cellular uptake. We have demonstrated that deficiency in the human equilibrative nucleoside transporter 1 confers high-level resistance to gemcitabine toxicity *in vitro* (Cancer Res, 1998; JNCI, 1999). No methods previously existed for assessing hENT1 abundance in solid tumors.

Frozen sections of 33 primary breast cancers were stained with a murine monoclonal antibody raised against a synthetic peptide derived from the large intracellular loop of hENT1. Antigen detection was performed using a goat-anti mouse antibody directly labeled with a polymer-perioxidase conjugate. Negative controls were provided by omitting the primary antibody. A pathologist, blinded to clinical characteristics and outcomes, scored hENT1 staining intensity on a 0-4 + scale. hENT1 staining was correlated with pathological and clinical characteristics.

hENT1 immunohistochemical staining intensity varied markedly among breast samples (score/n; 0/4; 1/5; 2/7, 3/14, 4/3). Tumor and breast tissue associated endothelial cells were universally positive, and provided an internal positive control. hENT1 staining did not correlate with pathological features (nuclear, architectural, mitotic or overall histologic grade; vascular invasion, tumor size, hormone receptor status) or clinical features (tumor stage, patient age, disease free survival, and overall survival).

hENT1 staining intensity is highly variable among human breast adenocarcinomas. Because hENT1 deficiency confers gemcitabine resistance *in vitro*, hENT1 immunohistochemical staining warrants study as a determinant of gemcitabine activity in metastatic breast cancer.

313 Prospective Analysis of the Oxygenation of Malignant Breast Tumors as a Predictor of Response to Primary Systemic Chemotherapy (PSC).

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PSC for operable breast cancer can be used as a tool for testing various biomarkers as predictive factors for tumor remission and as surrogates for disease prognosis. As only patients (pts) with a pathological complete response (pCR) seem to benefit from PSC concerning survival, there is a need for markers that predict pCR.

In a prospective analysis we polarographically measured intratumoral oxygen tension of T2 and T3 breast cancers using the Eppendorf histograph. The movement of the electrode through the tumor was guided by ultrasound. Then several core cut biopsies from the regions of oxygen measurement were performed.

In our institution 47 pts with breast cancer underwent measurement of oxygen tension and subsequent histological examination between 10/99 and 4/2000.

Age: > 50 years, n = 32; < 50 years, n = 15. Tumor size: T2, n = 44 (93%); T3, n = 3; 2 - 3 cm, n = 28 (60%); > 3 - 4 cm, n = 9 (19%); > 4 - 5 cm, n = 7 (14%); > 5 cm, n = 3 (7%). Histological Type: Invasive ductal, n = 36 (76%); invasive lobular, n = 6 (13%); other, n = 5 (11%). Hormone status: positive (ER and PR > 0), n = 35 (74%); negative, n = 12 (26%). Grading: G1, n = 12 (26%); G2, n = 21 (45%); G3, n = 14 (29%).

Preliminary results are available for 27 pts. pO2 was the same in the different tumor sizes with a mean of 15,3 mmHg. Median pO2 in G3 tumors was lower (9 mmHg) than in G2 (18 mmHg) and G1 (16 mmHg) tumors. Hormone receptor positive tumors showed a mean pO2 of 15 mmHg whereas negative tumors had a lower mean pO2 of 7 mmHg. In the group of pts who received PSC, those with a pCR (n = 2) had a mean initial tumor pO2 of 27 mmHg. In tumors with other responses (n = 9) mean initial pO2 was 12 mmHg.

In conclusion this first analysis confirmes the observation that oxygenation does not correlate with tumor size. In contrast there may be a correlation between pO2 and tumor grade and hormone receptor status. Further results must confirm these findings, especially the question of the predictive power of pO2 for tumor response to PSC.

314 6 Cycles of Epirubicin®/Taxotere® (ET) Versus 6 Cycles of 5FU/ Epirubicin®/Cyclophosphamide (FEC) as First Line Metastatic Breast Cancer (MBC) Treatment: Preliminary Results of a Randomized Phase II Trial.

Dieras V,[1] Tubiana-Hulin M,[2] Bougnoux P,[3] Bonneterre M-E,[4] Mayer F,[5] Delozier T,[6] Culine S,[7] Dohollou N,[8] Samak R,[9] Suissa J,[10] Bonneterre J.[4]
[1]Institut Curie, Paris; [2]Centre R Huguenin, Saint-Cloud; [3]CHU Bretonneau, Tours; [4]Centre O.Lambret, Lille; [5]Centre F.Leclerc, Dijon; [6]Centre F.Baclesse, Caen; [7]Centre Val D'Aurelle, Montpellier; [8]Polyclinique Bordeaux Nord; [9]Clinique St George, Nice; [10]Laboratoire Aventis, Montrouge, France.

Docetaxel and anthracyclines can be considered as the most active drugs in metastatic breast cancer and several phase I trials have shown the feasibility of ET combination. This study was conducted to evaluate the activity of ET (75/75 mg/m^2) combination versus a standard anthracycline based regimen (FEC 75) in first line metastatic breast cancer patients. Eligible females had histologically proven breast cancer with at least one bidimentionally measurable lesion, no prior chemotherapy for MBC, cumulative dose of equivalent doxorubicin ≤ 310 g/m^2, ECOG PS≤ 2, LVEF within the normal range, adequate haematologic, hepatic and renal functions, no adjuvant chemotherapy within the last 12 months. Between June 98 and April 2000, 102 patients (pts) were treated (ET:51/FEC:51), median age: 54 (23-73), PS 0/1/2: 68/ 31/3, median DFI: 47 months, and in respectively ET and FEC arms (%): adjuvant chemotherapy: 33/41, ≥3 organs: 23/20, liver metastasis: 43/47, lung metastasis: 37/ 27, bone involvement: 6/18. Median nb of cycles was 6 in both arms. To date, discontinuation for progression was observed in 15% of pts for ET and 31% for FEC. The main severe related NCI-CTC grade3-4 events were respectively in ET: %pt(%cy)/ FEC %pt(%cy) arms: neutropenia: 69(38)/61(28), febrile neutropenia: 23(7)/0, infection: 4(<1) (one toxic death: septic)/ 0, diarrhea: 4(<1)/0, nausea/ vomiting: 18(5)/14(3), asthenia: 4(<1)/6(3), G3 neurosensory: 4(<1)/0.

Conclusion: The feasibility of ET at a dose of 75/75 mg/m^2 is confirmed, the main toxicity is haematologic but less than 20% of these metastatic pts experienced dose adjustment or G-CSF support. Accrual is still ongoing and preliminary efficacy results will be presented during the meeting.

315 A Phase II Study of Weekly Docetaxel (TXT) as Primary Chemotherapy (PC) in Stage II and III Breast Cancer: Preliminary Results.

Estévez L, Cuevas JM, Antón A, Florián J, Lopez-Vega JM, Velasco A, and the Breast Cancer Investigation Spanish Group. GEICAM, Spain.

The aim of the study is to investigate the response rate (RR) of weekly TXT as PC in breast cancer patients (pts). Secondary objectives are to analyze Her2 as a predictive factor of response and the toxicity of treatment. From July 1999 to May 2000, pts with stage II or noninflammatory stage III breast cancer were included into the study. A biopsy tumor specimen was recovered before beginning PC to the analysis of Her2. TXT 40 mg/m2 was administered by 30 minutes intravenous infusion weekly for 12 weeks (wks) period, before definitive surgery. A 2-week break was inserted after the first 6 wks. Thirty-four pts have been included so far. Median age was 61 years (range 27-73). All pts had an ECOG 0. 15 pts IIA, 15 pts IIB and 4 pts IIIA. Pretreatment tumor size average was 4.6 cm. 16 pts were assessable to RR. Three of them withdrawn from study (1 for progression and 3 due to non-hematological toxicity), but they were included in this analysis. Overall RR was 81% (95% C.I. 62-100) with 8 complete response (50%) and 5 partial response (31%). Surgery has been performed in 10 pts and pathologic complete remission (pCR) was documented in 3 pts (30%). A total of 292 infusions have been recorded. Median number of infusion was 9.5 (range 2-12). In 9 pts (26%) the dose infusion was reduced along treatment. Grade 3-4 hematological toxicity was no documented. Grade 3-4 asthenia was observed in 6 pts (18%). Grade 3-4 skin reactions and nail disorders was also observed in 6 pts (18%) respectively. Weekly TXT seems to be an effective treatment as PC with minimal toxicity. Despite the small number of pts, we observed high pCR rate. Recruitment pts is still ongoing and updated of response and analysis of Her2 as a predictive factor will be presented.

316 Weekly Taxol (T) and Carboplatin (C) Regimen in Patients with Advanced Breast Cancer: A Phase II Study.

Loesch D, Robert N, Asmar L, Gregurich MA, Dakhil S.

Purpose: To determine the activity of weekly Taxol and Carboplatin given for 3 weeks followed by 1 week of rest as first-line therapy in patients (pts) with advanced breast cancer (ABC) by assessing response rate (RR), survival and safety.

Patients and Methods: Between November 1998 and August 1999, 100 pts with ABC were enrolled with intent to treat on this study. After premedication with dexamethasone 20 mg PO administered approximately 12 and 6 hrs before T, diphenhydramine 50 mg IV, and cimetidine 300 mg or ranitidine 50 mg IV, pts received in succession T 100 mg/m2 as a one hour infusion, followed by C at an AUC=2 by IV infusion over 30-60 minutes. ECOG performance status (0/1/2) was (53/41/6). Median age was 58.5 years (range=33-89 yrs), ER+/PR+ was 44%/41%, 43% had liver metastasis at baseline, 48% received XRT pre-treatment, and 61% had prior adjuvant chemotherapy of whom 37 (61%) received adriamycin. Initially, the Taxol® dose was 135 mg/m2 and Carboplatin AUC=2, however, due to increased toxicity in the first 20 pts (grp1) (50% of the pts had 25% dose reduction during the first 3 cycles), the weekly dose of T was reduced to 100 mg/m2 for grp2.

Results: Three pts withdrew consent before completing 2 cycles of treatment and were not evaluable for response. Responses were: 8 CR, 50 PR (58%) with a median time to response of 1.8 months (mos) (range, 1-5) with no differences in response between the 2 groups. Estimated median time to progression was 4.8 mos (range <1 - 17mos), 20 pts had stable disease. Estimated median survival for all patients is 13 mos (range 1 - 17 mos) with death mainly due to disease.

The incidence of grade > =3 neutropenia and leukopenia was 50% and 25% in grp1 compared to 32% and 11% in grp2, respectively. Grade > =3 Neuropathy 10%, and infection 5%, 3 pts had drug related sepsis (2 pts in grp1).

Conclusion: This open-label, multi-center study utilizing weekly T and weekly C gave a response rate of 58% which is comparable to the best response rates in ABC using chemotherapy. As pts become segregated into cohorts of HER-2+ and HER-2- groups for adjuvant therapy, non-anthracycline containing regimens will need to be considered for future adjuvant trials. With this high response rate and lack of cardiotoxicity, the weekly T and weekly C regimen should be given consideration for movement to the adjuvant setting.

Supported by Bristol-Meyers Squibb Oncology.

317 Phase II Trial of Weekly Docetaxel (Taxotere) Alone or in Combination with Trastuzumab (Herceptin) in Patients with Metastatic Breast Cancer.

Sparano JA, Malik U, Manalo J, Rajdev L, Sarta C, Hopkins U, Fineberg S. Albert Einstein Comprehensive Cancer Center, Montefiore Medical Center, Bronx, NY.

Weekly taxane therapy produces less myelosuppression and offers the potential for greater synergy with the anti-Her2 antibody Herceptin. (Semin Oncol 26 [Suppl 9]: 32-36, 1999)

Objectives/Methods: The objective of this study was initially to determine the efficacy of intravenous docetaxel (33 mg/M2 over 1 hour) in metastatic breast cancer given on a continuous weekly basis without planned treatment holidays, and was later modified to evaluate weekly docetaxel plus Herceptin (4 mg/kg, then 2 mg/kg IV) in patients with Her-2 overexpressing tumors (2+ or 3+ by the DAKO Herceptest).

Results: Patient characteristics: N=25; median age 54 years; at least 3 disease sites (9 [36%]); failed at least 1 prior chemo for metastases (22 [88%]) including either paclitaxel (6 [24%]) or docetaxel (8 [32%]) every 3 weeks for metastases.

Partial response occurred in 9 of 22 evaluable patients (41%), including 4 of 16 patients (25%) treated with docetaxel alone, 5 of 6 patients treated with docetaxel and Herceptin, and 4 of 10 patients (40%) treated with docetaxel alone who had no prior docetaxel for metastases. Patients received a median of 8 treatments (range 1-44) over 8 weeks (range 1-49), resulting in a median dose intensity of 33 mg/M2/week (range 25-33 mg/M2/week). Toxicity consisted of grade 1-2 lacrimation in 11 (44%), fatigue in 9 (36%), nail changes in 7 (28%), and fluid retention in 6 (24%). Grade 3-4 toxicites included hyperglycemia (N=4), granulocytopenia (N=4), neuropathy (N=2), mucositis (N=2), anemia (N=2), and infection (N=1).

Conclusions: These results are consistent with a previous report regarding weekly docetaxel with planned treatment holidays (6 weeks on/2 weeks off) (Burstein et al, Proc ASCO 1999, abstr 484), suggesting no therapeutic advantage for uninterrupted therapy. The activity and tolerability of weekly docetaxel and Herceptin are encouraging and merit further investigation. An updated analysis including 15 additional patients treated with a modified weekly schedule (40 mg/m2 3 weeks on/1 week off)will be presented.

318 Phase I-II Trial of Pegylated Liposomal Doxorubicin (Doxil) Plus Docetaxel (Taxotere) in Patients with Advanced Breast Cancer.

Sparano JA,[1] Malik U,[1] Wolff A,[2] Rajdev L,[1] Sarta C,[1] Hopkins U.[1] [1]Oncology, Montefiore Medical Center, Bronx, NY; [2]Winship Cancer Center, Atlanta, GA.

Doxil is associated with better tumor penetration in animals and less cardiotoxicity in humans than conventional doxorubicin. The objectives of this study were to determine the safety, feasibility, and efficacy of this combination. Doxil was infused over 60 minutes, followed 1 hour later by Taxotere given over 1 hour. Dose limiting toxicity (DLT) included febrile neutropenia (FN) or at least grade 3 toxicity during cycle 1. Patient characteristics: N=41; median age 52 range 32-83); stage IIIA/B (1/8[22%]) or IV (32 [78%]); bone metastases (20 [49%]). Results: The MTD for the 4-week schedule plus G-CSF for Doxil/Taxo tere was 40/75 mg/m2, and for the 3-week schedule without G-CSF was 30/60 mg/m2. DLTs consisted of FN (N=4), grade 3-4 mucositis (N=4), both (N=3),hypersensitivity (HSR) to Doxil (N=1), or severe neutropenia (* N=1; defined as DLT in first cohort only). The median number of cycles given was 5 (range 1-8). Only 2 patients (5%) had at least 10% decrease in the LVEF. The 30/60 q 3wk dose/schedule was well tolerated, with major toxicities including FN (13%) and grade 3-4 mucositis (20%), PPE (13%), and anemia (13%). Doxil-related HSRs were problematic early in the trial (30%), but were eliminated by stepped increment in the infusion rate. Objective response occurred in 6 of 8 patients (75%) with stage III disease, and 18 of 33 patients (55%) with stage IV disease. The median response duration for patients with stage IV disease was 7 months (range 2+ - 14+ months). Conclusions: The recommended dose of this combination without G-CSF is Doxil 30 mg/M2 and Taxotere 60 mg/M2 every 3 weeks. The lower cardiotoxicity of Doxil renders this Doxil/Taxotere regimen logical for combination with trastuzumab (Herceptin), which is being evaluated in an Eastern Cooperative Oncology Group trial (E3198).

Doxil mg/M2	Taxotere mg/M2	Schedule	G-CSF	No. Pts	No. DLTs
30	75	4 wk	No	3	2 *
30	75	4 wk	Yes	3	0
40	75	4 wk	Yes	3	0
45	75	4 wk	Yes	5	4
40	75	4 wk	Yes	12	6
30	60	3 wk	No	15	1

319 Randomized Trial on Dose-Intense Adjuvant Chemotherapy with Epirubicin and Cyclophosphamide in High-Risk Breast Cancer Patients.

Thomssen C,[1] Untch M,[2] Behrens K,[1] Kahlert S,[2] Sattler D,[3] Oberlechner E,[4] Kuhn W,[3] Lebeau A,[2] Dettmer P,[3] Konecny G,[2] Jaenicke F.[1] [1]Ob/Gyn, University Hospital, Hamburg; [2]University Hospital Großhadern, Munich; [3]Technical University, Munich; [4]Hospital Landshut, Germany.

Introduction: Patients with tumor infiltration of 10 or more axillary lymph nodes or extracapsular node infiltration have an unfavourable prognosis. We report on the results of a randomized prospective multicentre trial on dose-intense adjuvant chemotherapy.

Methods: Between 1993 and 1998, 182 patients were recruited and randomly assigned to dose-intense adjuvant chemotherapy (diEC 120/600 *4, q14d) in comparison to a conventional regimen (EC 90/600 *4, q21d => CMF*3). In 180 evaluable patients, 63 recurrences and 44 deaths were observed. Median follow-up of surviving patients is 42,1 months (2,8 to 78,0 months).

Results: In univariate analysis, patients treated with diEC had an estimated 63.7% 4yrs-disease-free survival (DFS), patients with conventional therapy 47.3% (p=0.0729). By diEC, risk of recurrence might be reduced by 36.5%. The 4yrs-OS (overall survival) was 80.9% and 68.5%, respectively (p=0.0487). Retrospective subgroup analyses showed benefit by diEC particularily for patients with less than 10 axillary lymph nodes and concomitant extracapsular tumor infiltration (n=88; 4yrs-DFS 83.8% vs. 59.8%; p=0.0304; 4yrs-OS 90,6% vs. 70.9%; p=0.019). In patients with more than 10 tumor infiltrated axillary lymph nodes, no benefit by dose-intensification could be demonstrated.

Conclusion: Although the study was not designed to perform subgroup analyses, it seems that in patients with more than 10 tumor infiltrated lymph nodes, dose-intensification does not result in prolonged survival. In contrast, in patients with less tumor extension, but presumably high-risk biology as indicated by extracapsular disease, dose-intensification of epirubicin and cyclophosphamide might improve the course of the disease.

320 Oral Single-Agent Estramustine (EM) Is Active in Advanced Breast Cancer (ABC) after Failure with Anthracyclines and Taxanes.

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EM is an oral non-hematotoxic agent which depolymerizes tubulin, a mechanism of action that has been revisited during the last decade. The aim of the study was to assess its tolerance and efficacy in ABC after failure with the usual regimens. Pts with PS<=3 (ECOG) and measurable disease having received at least 1 line of chemotherapy for ABC (including taxanes or anthracyclines) were eligible. EM was given daily at a dose of 10 mg/kg until disease progression or unacceptable toxicity. Forty pts were included between June 1998 and December 1999. Pts had previously received 1 to 8 regimens (median = 2) for ABC and the median age was 54 years (34-78). Adverse events leading to early interruption of EM were: gr. 2 allergy (n=1), gr. 2-3 nausea (n=6), deep-vein thrombosis (n=1), gr. 3 sepsis (n=1). One pt died at 24 weeks from pulmonary embolism, and another at 16 weeks from unknown cause. Other toxicities were: gr. 1-2 nausea (n=5), vaginal discharge (n=1), uterine bleeding (n=1). Seven objective responses were seen (17.5%) with a time to failure (TTF) ranging from 14 to 52+ weeks (median=24). All but one of these pts had visceral disease (liver : n=4, lung : n=2, CNS : n=1). In 10 other pts (25%), disease remained stable with a median TTF of 27 weeks (16-50); 6 of them experienced relief of symptoms and improvement of their PS with a concomitant decrease in CA15-3 levels. It can be concluded that daily oral EM is an active agent in ABC with a very favorable tolerance profile since no other life-threatening toxicities than thrombo-embolic complications are observed in our study. Further development of EM is warranted in ABC including combination regimens with other tubulin-active agents.

321 Phase II Trials of Carboplatin/Docetaxel and Carboplatin/Docetaxel/Traztuzumab as First Line Therapy for Metastatic Breast Cancer.

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Potential preclinical synergy with traztuzumab has reawakened interest in platinum analogues in the treatment of metastatic breast cancer. Based on phase I data from our institution, we developed phase II trials of carboplatinum/docetaxel (CD) and carboplatin/docetaxel/traztuzumab (CDT) for the first-line treatment of metastatic breast cancer. As of June 1, 2000, 19 patients have enrolled in our phase II trial of CD. Median age was 49 (29-62) and median ECOG PS was 0 (0-1). Sites of disease included lung (8/19, 42%), liver (4/19, 21%), bone (8/19, 42%), LN (5/19, 26%), and chest wall (3/19, 16%). Carboplatinum was given at an AUC 6 and docetaxel at 75 mg/m2 IV q3weeks (with standard premedication) for at least 6 cycles or until progression. Median number of cycles given was 4 (2-8). Severe toxicities were primarily hematologic. Grade 4 neutropenia with fever occurred in 3 of the first 12 patients treated and grade 3 or 4 thrombocytopenia occurred in 5 of the first 12 patients treated, necessitating a dose reduction of the carboplatin to an AUC of 5 for all patients. No grade 3 or 4 thrombocytopenia was seen at a carboplatin AUC of 5. Overall grade 3 or 4 toxicities for all patients were neutropenia (16/19, 84%), thrombocytopenia (5/19, 26%), and fatigue (2/19, 11%). 15 patients are evaluable for response, with an overall response rate of 60% (9/15) and a complete response rate of 20% (3/15). A parallel phase II trial of CDT is also underway, with carboplatin AUC 5, docetaxel 75 mg/m2 IV q3w and traztuzumab 4 mg/kg IV wk 1 and 2 mg/kg IV weekly thereafter until disease progression. 6 women (ages 36-54, PS 0-1, Her 2 Neu 2+ or 3+ by immunohistochemistry) are currently enrolled. No dose reductions or delays have been recorded in 34 cycles of therapy to date. Toxicity is primarily grade 3-4 neutropenia in all 6 patients. 5 patients are evaluable for response, with 4/5 patients responding (2 CR, 3 PR). Although these results are preliminary, CD and CDT have promising activity in MBC. Accrual continues to both studies, and updated data on response and toxicity will be presented.

322 Phase II Study of Gemcitabine and Cisplatin in Patients with Metastatic Breast Cancer (MBC) and Failure on Prior Chemotherapy: A North Central Cancer Treatment Group Trial.

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Recent reports have shown that gemcitabine is an active agent in breast cancer. The combination of gemcitabine and cisplatin has shown synergistic activity in the laboratory and has been given safely to patients with non-small cell lung cancer. We undertook this phase II study of these agents as treatment for women with MBC who had progressed following at least one prior chemotherapy regimen for metastatic disease. Prior treatment must have included an anthracycline or taxane in either the adjuvant or metastatic setting. Eligibility criteria: measurable indicator lesion, ECOG PS 0-2 and adequate hematologic, renal and hepatic function. We utilized a two-stage design where 20 patients were to be enrolled in the first stage. If ≤3 responses were observed the trial would be stopped otherwise another 30 patients would be accrued to a second stage. This would allow the detecting a true response rate of ≥0.30 at the 0.05 significance level. Twenty-one patients (+1 ineligible) were entered in the first stage. Patients received gemcitabine 1000 mg/m^2 IV + cisplatin 25 mg/m^2 IV day 1, 8, 15 of a 28 day treatment cycle. Patient characteristics include: PS 0-1 in 90%, visceral-dominant disease in 90%, two prior chemo. regimens in 81%, and prior XRT in 48%. Six responses were seen (1CR, 5PR) for a response rate of 29% (95%CI 11-52%). Time to progression was 7.1 months. Hematologic toxicities include neutropenia (38% grade 3, 43% grade 4), and thrombocytopenia: (24% grade 3, 38% grade 4). ≥Grade 3 nonhematologic toxicities (# patients) include: nausea (3), vomiting (2), diarrhea (1), constipation (1), stomatitis (1), lethargy (1), neuro-motor (1), and dyspnea (1). Given the response rate in the first stage accrual proceeded to a second stage which has been completed. Due to the significant hematologic toxicities in the first stage gemcitabine doses were lowered to 800 mg/m^2. Updated results for the entire cohort will be presented.

323 Low Weekly Dose of Paclitaxel (P) in the Treatment of Metastatic Breast Cancer (MBC) Patients (pts). A Phase II Study.

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P is one of the most active drugs in MBC and a weekly schedule, at a median dose-intensity of 91mg/m2 (Seidman, JCO 1998), was effective and with less side effects in comparison to a 3-week schedule. Eighteen heavely pretreated pts were enrolled onto the study from February 1999 to March 2000. P was administered at a dose of 60-70 mg/m2 by 1-hour iv infusion. Treatment was planned to continue until disease progression or toxicity. The median age of pts was 64 yrs (range 33-75), the median PS 1 (range 0-1). Prior therapy included: adjuvant only 3 pts, metastatic only 10 pts, or both 5 pts. Ten pts had received two or more regimens for metastatic disease. Twelve pts had received prior anthracycline therapy, 8 of whom were considered anthracycline-resistant. Six pts had received prior taxanes. Seventeen pts had metastases at more than one site and 9 had metastases at more than 3 sites. Fifteen pts (83%)had visceral metastases. A total of 248 weekly infusions were delivered with a median of 11 (range 5-34) infusions per pt.Two pts are too early and 16 pts were evaluated for response. We obtained: PR 3 pts (19%;95%CI 0-37%), SD 5 pts (31%) and PD 8 pts (50%). Two PR were observed in anthracycline-resistant pts. Median response duration and time to progression were 38 weeks (range 12-60) and 13 weeks (range 6-47), respectively. Treatment was well tolerated. Side effects included alopecia, fatigue, and nausea. Haematologic toxicity was mild. Dose omissions occurred in 3 pts because of grade 2 neutropenia. One pt in PR came off study secondary to peripheral neuropathy after 9 weeks but she had been treated with cisplatin and docetaxel. She was started on anastrozole and she is still in response. Although we utilized a lower dose of P, weekly therapy with 1-hour infusion confirms a substantial degree of activity also in heavily pretreated pts, with a favorable toxicity profile. We are still recruiting patients.

324 Monthly Cisplatin (C) and Gemcitabine (G) as Second Line Chemotherapy for Patients with Advanced Breast Cancer.

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Introduction: Gemcitabine is a nucleoside analogue and has yielded promising results in the therapy of stage IV breast cancer. Both G and C are active against several solid malignancies, including breast cancer. Experimental data, both in vitro and in vivo, suggest that the combination Gemcitabine-Cisplatin should interact synergistically. This phase III study with Gemcitabine plus Cisplatin (G/P) in patients with metastatic breast cancer (MBC) progresssing despite previous anthracycline-containing chemotherapy. Patients and Methods: Fortyone eligible patients have been entered on study evaluable for toxicity and response. Median age 61.5 ±9.3 yrs, range 33-75, performance status (WHO) 0: 23,1:15,2:3, and adequate hematologic, renal, hepatic functions, written informed consent. Gemcitabine (G) was administered on days 1,8, and 15 as a 30 min. IV infusion at doses 1200mg/m2 and Cisplatin (C) 50mg/m2 on d 1 of 28 days cycle. Results: The overall response rate (RR) is 48,78% with CR:4/41, 9.75%; PR:16/4, 39,03%; SD: 10/41, 24, 39%; and PD:11/41, 26,82%. Median time to response is 1.8 months (1.1-3.8), median duration of response is 10.6 months (5.412.7), median time to progression (TTP) is 5.2 months (3.5-8.7) grade 3-4 toxicities included thrombocitopenia (47%); neutropenia (48%); anemia (42%); nausea and vomiting (17%); alopecia (77%); nephrotoxicity (9%) and neuropathy (11%). There were no episodes of neutropenic fever or thrombocytopenia-related bleeding. Red cell and platelet transfusions were required by 35% and 10% of patients, respectively. Conclusion: Gemcitabine and Cisplatin is highly active in relapsed breast cancer with a moderate toxicity profile.

325 Paclitaxel (P) Maintenance Treatment Following First Line Chemotherapy with Anthracyclines Plus Paclitaxel in Metastatic Breast Cancer (MBC): Preliminary Results from the Italian MANTA 1 Study.

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Despite the high response rate obtained in patients with metastatic breast cancer the association of taxanes and anthracyclines seems not to improve the progression free and overall survival of these patients. The possibility of mantain the response obtained prolonging the duration of chemotherapy may represent a good strategy and this approach is becoming of common use in clinical practice. With these premises we are performing a phase III multicenter trial comparing PFS and OS between maintenance therapy with paclitaxel or no treatment. As first line chemotherapy for MBC patients receive one of the following regimens: a) Epirubicin 90 mg/sqm + P200 mg/sqm (3 hr) q.21 days; b) Doxorubicin (D) 50 mg/sqm 16 hrs before P 200 mg/sqm (3 hr) q.21 days; c) D50 mg/sqm d. 1 + P 200 mg/sqm d.2 (3hr) q.21 days, for 8 courses. Responding patients are randomized to receive further 8 courses of P 200 mg/sqm q.21 or no treatment. At present 250 patients have been enrolled and 118 have been randomized. Patients characteristics are as follows: median age 55 yrs (range 30-73), median PS 0 (0-2), 29% of the patients wre premenopausal, 58% were ER+, 50% had received prior adjuvant CT and 19% hormonal treatment for mts disease; 29% of the pts were metastatic ab initio, dominant metastatic sites: viscera in 64% of the pts, bone 21%, soft tissue 15%; number of metastatic sites: 1 37% of the pts, > 3 23%. At randomization 51.5% of the pts received P and 48.5% no further CT. 10% of the pts have not been randomized for progressive disease and 4.5% for unacceptable toxicity, while 6% of them refused the treatment. In addition data on the compliance to maintenance CT, toxicities, PF and OS will be presented.

326 Weekly Docetaxel in Advanced Breast Cancer with Progression after Treatment with Anthracyclines.

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Docetaxel administered every three weeks has shown to be the most active single agent in the treatment of metastatic breast cancer. The aim of the study was to assess the efficacy and safety of weekly docetaxel, in advanced breast cancer with failure to previous treatment with anthracyclines. 47 patients with measurable disease, no more than 2 prior regimens for the disease and with disease progression after previous anthracycline treatment were recruited to participate in this phase II trial. The treatment scheme was docetaxel administered at a starting dose of 40 mg/m2 weekly for 6 weeks, followed by a 2-week rest period (1 course). Dose reduction was required in case of G II neurotoxicity, G III non-haematological toxicity or febrile neutropenia and one week delay was required for ANC<1000. Premedication with oral dexamethasone (8 mg) was administered the night before, morning of, and evening after each docetaxel administration. After including the first 13 patients, the protocol was amended for a reduction of the starting dose to 36 mg/m2. Mean age was 60.1 years (range 33-77); performance status: ECOG0: 41%, ECOG1: 34%; ECOG2: 25%. Metastatic sites were 21.3% liver, 46.8% bones, 8.5% nodes, 31.9% skin, 23.4% lung, 6.4% others. Total number of courses administered was 102 (mean 2.2 courses per patient). Relative docetaxel dose intensity was 89%. 45 patients were evaluable for response. 13.3% (6/45) CR; 22.2% (10/45) PR; ORR 35.6% (16/45), (95%CI 21.6-49.6); 33.3% (15/45) SD; 31.1% (14/45) DP. One pt. presented neutropenia G IV and 3 pts. neutropenia G III. No other G III-IV haematological toxicities were observed. The most frequent non-haematological toxicities were asthenia G I-II 15 pt, G III-IV 5 pt, nausea GI-II 18 pt, nail disorders GI-II 10 pt, GIII-IV 4 pt, vomiting G I-II 9 pt. Median time to disease progression was 246 days (95%CI: 140-257) and median overall survival 282 days (95%CI: 230-334). Weekly docetaxel is well-tolerated and appears to have encouraging efficacy in antracycline-pretreated patients with advanced breast cancer.

327 Results of Two Open-Label Multicentre Pilot Phase II Trials with Herceptin® in Combination with Docetaxel and Platinum Salts (Cis- or Carboplatin) (TCH) as Therapy for Advanced Breast Cancer in Women Overexpressing HER2.

Nabholtz JM, Crown J, Yonemoto L, Tannenbaum S, Klimo P, Patel R, Fumoleau P, Sanchez J, Prady C, Villa D, Ellis E, Pegram M, Lindsay MA, Slamon D, and the Breast Cancer International Research Group (BCIRG). UCLA, Los Angeles, CA.

Based on published and confirmed biologic data demonstrating true pharmacologic synergy between Herceptin® (trastuzumab) and either docetaxel or platinum analogs in terms of antitumor activity, as well as the cardiac toxicity associated with anthracycline-Herceptin®-based combination regimens, we proceeded with 2 pilot TCH phase II trials, one combining docetaxel/Herceptin® and carboplatin (TCH1) and one combining docetaxel/Herceptin® and cisplatin (TCH2). **Objectives:** The primary objectives of these pilot studies were to evaluate the efficacy and safety of TCH as therapy for patients (pts) with HER2-positive advanced breast cancer. Secondary objectives were duration of response, time to disease progression and survival. **Patients and methods:** A total of 90 HER2-positive (immunohistochemistry and/or fluorescence in situ hybridization [FISH]) stage III/IV breast cancer pts were planned to be treated (TCH1: 50 pts; TCH2: 40 pts). Eligible patients are treated with Herceptin® 4mg/kg on day 1 (90-min IV infusion) followed by 2mg/kg weekly (30-min IV infusion), plus docetaxel 75mg/m^2 (1-h IV infusion) and either cisplatin 75mg/m^2 (1-h IV infusion) or carboplatin (AUC of 6) on day 1 every 3 weeks (for 6-8 cycles). All patients are to continue weekly Herceptin® until disease relapse. **Results:** As of today, 37 patients have been accrued in the studies. Toxicity in 22 pts in the TCH1 pilot, who to date have received a total of 79 cycles of TC and 223 cycles of H, has been acceptable with 13 patients developing grade 3/4 neutropenia and only 1 episode of febrile neutropenia. Gastrointestinal toxicity was mild (1 pt with grade 3 mucositis). No significant cardiac¸ neurologic, renal or hepatic toxicity was observed. To date, with assessment of 12 patients, objective responses were seen in 7. All FISH-positive patients had objective responses including 2 CRs, 1 of which was a confirmed pathologic CR. 5 patients with stable disease were FISH negative. Updated safety and efficacy data on the total patient population will be presented at the meeting. **Conclusions:** These pilot studies represent the clinical basis for the BCIRG TCH phase III program in first-line metastatic and adjuvant treatment of HER2/neu-positive breast cancer patients.

328 Taxotere and Adramicin-Cyclophosphamide (AC) in High Risk Localized Breast Cancer.

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The objectives of this study were to evaluate the efficacy of taxotere administered following AC in patients with high risk breast cancer. 33 patients with previous surgical treatment for the disease were included in this study. Mean age was 49.2 years (range 31-67); baseline performance status was ECOG0: 77.8% and ECOG1: 22.2%. Patients received 4 cycles of A (60mg/m2 i.v. bolus) and C (600mg/m2 i.v. bolus) on day 1 followed by docetaxel 100mg/m2 intravenously over 1 hour every three weeks for 4 cycles. A total of 128 cycles of AC and 121 of docetaxel were given. Dose reduction was needed in 2 cycles for AC and in 11 cycles for docetaxel. Relative dose intensity was 0.76 for A, 0.77 for C and 0.91 for docetaxel. Only 2 patients had progressed at the time of this analysis. Toxicity is described per patient and separately for AC and for docetaxel. No grade III/IV haematological toxicity was described for neither AC nor for docetaxel. 31.3% of patients had G I-II and 6,3% had G III nausea and 18% G I-II stomatitis for AC. Nausea was described for docetaxel in 31,3% GI-II and 6,3% G III of patients, together with neuromotor disorders 34,4% GI-II, 3,1% G III, asthenia 25% G I-II, 6,3% G III, nail disorders 28,1% G I-II and stomatitis 21,9% G I-II 6,3% G III. This treatment has shown efficacy in preventing progression in high risk localized breast cancer, with no major haematological toxicity reported.

329 Docetaxel and Doxorubicin as First-Line Chemotherapy for Metastatic Breast Cancer (MBC) after Adjuvant CMF Failure. A Multicenter Nonrandomized Study.

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Doxorubicin (A) and Docetaxel (D) are highly active drugs in MBC. They have different mechanism of action, relative non-cross resistance and non-overlapping toxicity.

Patients and methods: In 11 centres in Poland, 35 patients (pts) in first line of chemotherapy for MBC, were treated with doxorubicin (Dox) 50mg/m2 and docetaxel (Taxoter) 60mg/m2, every 3 weeks.

The most important inclusion criteria was as follows: PS 0-2, measurable disease, normal renal, hepatic, bone marrow and cardiac function, prior adjuvant chemotherapy with CMF. Written informed consent form was obtained. Median of disease free time was 24 mths (range 4-204 mths). The pts median age was 52 years (range 35-67). One localisation of disease was in 15 pts, and multiple sites were seen in 20. Localisation of metastatic lesion was as follows (% of pts): lungs 46%; liver 31%; soft tissue 71%; other sites 37%. All pts were evaluable for toxicity (NCI), 34, for response. The total number of cycles was 215 (mean - 6 cycles).

Toxicity: febrile neutropenia was observed in 2% of cycles (14% of pts); grade 3/4 neutropenia in 41% of cycles (88% of pts); grade 3/4 thrombocytopenia in 1,8% of cycles (4% of pts); grade 3 infection in 0,6% of cycles (3% of pts) ; grade 2 alopecia in 77% of pts.

Response rate: complete response (CR), 26%; partial response (PR), 41%; stable disease (SD), 24%; progressive disease (PD), 9%; overall response (OR), 67%.

Median time to progression was 35 weeks. Median overall survival time was at least 54 weeks.

CONCLUSION: The combination of doxorubicin and docetaxel is a well tolerated and very active regimen for the treatment of MBC as first-line chemotherapy (OR - 67%, CR - 26%, median time to progression 35 weeks) after adjuvant CMF.

330 A Pilot Study of Docetaxel (D) and Vinorelbine (V) in Metastatic Breast Cancer (MBC).

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The standard chemotherapy in MBC remains anthracycline-containing regimens. There is an important group of patients (pts) in which anthracycline is contraindicated. D and V have shown antitumor activity in MBC refractory to anthracyclines, so we have conduced a pilot study to assess tolerability with a combination of these two drugs.

Patients and methods: The most important inclusion criteria were as follows: PS 0-2, measurable or evaluable disease, normal renal, hepatic, bone marrow and cardiac function, previous anthracycline containing chemotherapy. Written consent form was obtained.

12 pts received D 75 mg/m2 over 1 hour, followed by V 30 mg/m2 over 8-10 minutes on day 1 every 21 days. All pts received this combination as second line chemotherapy. All had received previous anthracycline containing regimens - 4 as neoadjuvant therapy, 8 -as first line metastatic chemotherapy. Mean age was45,7 years (range 36-61).

Localisation of metastatic lesion was as follows (% of pts): lungs 42%; liver 42%; soft tissue 42%; other sites 25%. All pts were evaluate for toxicity and for response. The total number of cycles was 59 (range 2-8).

Toxicity: neutropenic fever was observed in 33% of pts; grade 4 neutropenia in 42% of pts; grade 2 anaemia in 8% of pts; grade 2 diarrhoea in 17% of pts; grade 2 fluid retention in 8% of pts; grade 2 myalgia in 25% of pts; grade 2 infection in 17% of pts; grade 3 mucositis in 33% of pts; peripheral neuropathy grade 1 only, was observed in 2 pts (17%). 4 pts required G-CSF (33%).

Response rate: complete response (CR), 8%; partial response (PR), 58%; stable disease (SD), 26%; progressive disease (PD), 8%; overall response (OR), 66%.

Median time to progression was 17,3 weeks. Survival data will be presented in December 2000, with a longer follow-up.

CONCLUSION: A combination of one day D+ V chemotherapy is active regimen in MBC, the main toxicity being neutropenia. This combination seems to be active but toxic in pts previous treated with anthracycline containing regimens. Phase II study is ongoing.

331 Capecitabine: An Active and Well Tolerated Treatment Option for Patients with Metastatic Breast Cancer Recurring after Taxane-Containing Chemotherapy. Results of a Multicenter Phase II Trial.

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Capecitabine (C) is an oral tumor-activated fluoropyrimidine carbamate which has shown promising activity in patients (pts) with heavily pretreated metastatic breast cancer (MBC).

In this study we investigate the activity and toxicity of C after pretreatment with either paclitaxel or docetaxel. At study entry, pts have to have MBC with documented progression after a taxane containing chemotherapy, adequate bone marrow, hepatic and renal functions, measurable or evaluable lesions, and no prior chemotherapy with 5-FU continuous infusion. Treatment consists of C 1250 mg/sqm bid orally for 14 days followed by 7 days rest (=1 cycle). 97 pts have been entered so far. The median age is 55 years (range 36 to 77), and the median Karnofsky-index is 90 % (range 60 to 100). Pretreatment included anthracycline-based chemotherapy in 91 % and taxanes in 100 % (51 % paclitaxel, 45 % docetaxel, 4 % both taxanes). 77 and 68 pts are evaluable for toxicity and response, respectively. Median number of cycles administered is 4 (range 1 to 15). Toxicity was generally low with grade 1 or 2 hand-foot syndrom (40 %), nausea/vomiting (38 %), diarrhea (21 %), and lethargy (18 %). Grade 3 toxicity consisted of hand-foot syndrom in 14 %, and nausea/vomiting in 6 % of pts. Responses so far included 2 CR (3 %), and 15 PR (22 %). Disease stabilisation occured in another 47 % of pts accounting for an overall tumor control rate of 72 %. Progressive disease as best response was seen in 19 pts (28 %).

Conclusions: Capecitabine is a well tolerated oral chemotherapy with high activity in MBC after intensive pretreatment including paclitaxel and docetaxel. Recruitement is ongoing for a total of 140 pts.

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332 Phase II Study of Weekly Docetaxel in the Treatment of Metastatic Breast Cancer.

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Introduction: This study was conducted in order to investigate the efficacy and toxicity of weekly docetaxel administration in patients with metastatic breast cancer. Patients and methods: Thirty-seven women have been treated with a 1-hour infusion of docetaxel at 40 mg/m2 weekly for three consecutive weeks followed by one week rest. All patients were assessable for toxicity; five patients who were not assessable for clinical response, time to progression (TTP), and overall survival (OS) because of early treatment failure but are included in an intent-to-treat analysis. Results: No patient showed complete response whereas 14 patients had partial responses, which accounted for 38% of objective response rate. In addition, three patients (8%) had stable disease over 6 months. Clinical responses were achieved at a median of 3 cycles (range 1-4 cycles). Patients received a median of 4 cycles (range, 1-9) with a median dose intensity of 28mg/m2/week (range, 22-30). A median relative dose intensity was 0.95 (range, 0.73-1.0). Median TTP and median OS was 4 months and 10 months, respectively. The regimen was generally well tolerated. There was no febrile neutropenia with 19% of grade 3/4 neutropenia including only one grade 4. Non-hematologic toxicity, however, such as asthenia/fatigue, nail damage, eye tearing/conjunctivitis or auditory disorder were seen with repetitive cycles. Conclusion: Weekly docetaxel administration is active and feasible regimen for metastatic breast cancer patients.

333 Weekly Combination of Taxol, 5-Fluorouracil and Leucovorin (TFL) in Advanced Breast Cancer Patients (pts).

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Introduction: The optimal dose and schedule of paclitaxel is not yet defined. Preclinical data have suggested that duration of exposure is an important factor in the cytotoxic activity of this drug. A method to produce extended comulative exposure is the frequent drug administration, such as by the weekly schedule. **Purpose:** To evaluate the efficacy and toxicity of the TFL combination administered as weekly schedule. **Patients and Methods:** From May 1998 to February 2000 49 pts with advanced pretreated breast cancer were included in this study. Patients characteristics: median age: 54 (range 37-70); PS 0-1: 44 pts, PS 2-3: 5pts; pre/postmenopausal: 5/44 pts; ER +/-: 25/14 pts; ER unknown: 10 pts. Metastatic sites: bone: 16 pts; soft tissues: 14 pts; viscera: 19 pts. All patients have received prior anthracycline chemotherapy regimen. The TFL combination was the 1st metastatic line treatment in 12 pts, the 2nd line in 26 pts and the 3rd line in 11 pts. Treatment Plan: Paclitaxel 80 mg/m^2/wk as 1-hour iv infusion, 5-FU 300 mg/m^2/wk, Leucovorin 10 mg/m^2/wk, G-CSF 150 µg/m^2 on days 2 and 4. All pts received standard premedication. Treatment was planned to continue for 24 consecutive weeks in absence of progressive disease; median number of courses was 20 (range 4-24) and median dose-intensity was 97% (range 79-100). **Results:** All patients and 727 weekly corses (c) were evaluated for toxicity; no grade 3-4 toxicity was observed. One patient experienced hypersensitivity reaction with therapy discontinuation. Forty-seven of 49 pts were evaluable for response: CR 3 pts (6.5%), PR 21/47 pts (45%), SD 6/47 pts (12.5%) and PD 17/47 pts (36%). ORR was 24/47 pts (51.5%). Median duration of response: 8.5 months; Time to Progression: 6 months. **Conclusions:** The weekly TFL combination is active and well tollerated in athracycline-pretreated breast cancer pts.

334 Capecitabine Named Patient Programme for Patients with Advanced Breast Cancer: The UK Experience.

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87 patients with advanced breast cancer received Capecitabine in a UK open access programme and have been analysed for response and toxicity. Median age was 52.9 (range 30-95). A median of 5 cycles were given. 53% of patients had visceral disease and median number of sites of disease was 1. 64% had previously received anthracyclines, 28% taxoids and 6.9% infusional 5-FU.

Event	Grade 1 (%)	Grade 2 (%)	Grade 3 (%)	Grade 4 (%)
Neutropenia	2.3	1.2	2.3	1.2
Thrombocytopenia	4.6	2.3	1.2	0
Mucositis	8.1	5.8	2.3	0
Fatigue	9.2	4.6	3.5	1.2
PPE	11.5	11.5	9.2	0
Diarrhoea	21.8	6.9	3.5	2.3
Nausea	18.4	6.9	1.2	0
Vomiting	8.1	3.5	1.2	0

Dose reductions occurred in 29% of patients (9% of cycles). The mean dose intensity was 94.5%. There were 3 complete responders, 14 partial responders and the total objective response rate was 19.5%. Stable disease was achieved in 39% and progression was seen in 30%. We conclude that Capecitabine was well tolerated and active in extensively pre-treated patients with advanced breast cancer. Toxicity was manageable at the recommended dose of 1250mg/m^2 bd for 14 days q 21 days.

335 Taxane-Based Chemotherapy(TBC)-Induced Colitis in Breast Cancer Patients: M. D. Anderson Cancer Center Experience.

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Potentially fatal colitis associated with docetaxel-based chemotherapy has been observed in patients with metastatic breast cancer (Lancet 355, 2000). To appraise the frequency of this complication, we retrospectively reviewed the records of all breast cancer patients on TBC who were admitted to M. D. Anderson Cancer Center with gastrointestinal complications (GIC) between January 1997 and August 1999. During this period, 835 patients received TBC. Seventy-one admissions (63 patients, 7.5%) resulted from TBC-induced GIC. The main complaints for admission include nausea and vomiting, 43; abdominal pain, 35; diarrhea, 34 (7 were bloody); stomatitis, 32; and constipation, 10. Neutropenia and/or fever were associated with 51 admissions, while the rest of the patients were afebrile and non-neutropenic. Fourteen of the admitted patients (22%), 10 with docetaxel-based chemotherapy and 4 with paclitaxel-based chemotherapy, were admitted 16 times with a diagnosis of colitis. All patients with colitis presented with abdominal pain, with or without other symptoms, as described above. One patient died, and the autopsy revealed cecal ulceration. Colitis was found by CT scan of abdomen and pelvis during 12 admissions (10 patients); 2 of them were further confirmed by endoscopy and another 2 by exploratory laparotomy for bowel perforation. Three additional admissions produced a clinical diagnosis of colitis but without CT scan or endoscopic confirmation. Follow-up revealed that recurrences were seen in 2 patients upon re-challenge with the same dose of TBC; dose reduction in 7 patients (3 of paclitaxel and 4 of docetaxel) avoided the recurrence of colitis; no recurrence was observed after TBC was discontinued. This indicates that TBC can cause severe, occasionally fatal colitis in breast cancer patients. Taxane-related colitis is dose related, and dose reduction or discontinuation of the taxane successfully prevents recurrence.

336 Advanced Breast Cancer: Combination Chemotherapy with Doxorubicin and Weekly Docetaxel Treatment.

Méndez M,[1] Quiben R,[1] López P,[1] Enrech S,[2] Menéndez P,[2] Sancho JF,[2] Casinello J,[3] Domínguez S,[4] Palomero MI,[5] Perez-Manga G.[5] [1]H. de Móstoles, Madrid; [2]H. Gómez Ulla, Madrid; [3]H. Guadalajara; [4]Txagorritxu, Vitoria; [5]H. Gregorio Marañón, Madrid, Spain.

The objective of the present Phase II, multicentre and prospective study, was to evaluate the efficacy and toxicity of combination chemotherapy with doxorubicin and weekly docetaxel in patients with advanced breast cancer. For this purpose 49 patients with measurable and/or evaluable disease were recruited, all of them with an ECOG performance status ≤ 2, normal hematological, renal and hepatic function, and life expectancy ³ 3 months. Patients received doxorubicin (50 mg/m2 intravenously over 15 minutes) every four weeks followed, after 1-hour, by docetaxel (36 mg/m2 intravenously over 30 minutes) weekly on days 1,8 and 15 followed by a 1-week rest. Treatment was administered until 6 cycles were completed in 47.2% patients, 22.2% were removed from the study before the 6th cycle because of disease progression, and 5.6% were removed due to toxicity. Most patients (41.2%) presented T=2, N=1 and M=0 at baseline, and ECOG = 0 (63.6%). The most frequent histology was infiltrating ductal carcinoma (87.5%), the mean time from the first diagnosis was 39 months (SD = 39.2) and 91.7% of the patients had undergone surgery, 41.7% had received radiotherapy and 66.7% chemotherapy, previously. Forty patients were assessable for efficacy. The mean number of cycles administered was 3.96 and at the end of the study an overall response rate of 70% (95% CI: [56%, 84%]) was observed, 7 complete responses and 21 partial responses. Seven (14.6%) patients showed stable disease and five (10.4%) disease progression. The most frequent grade III/IV toxicities were alopecia (60.4%), neutropenia (29.2%), asthenia/fatigue (20.8%), febrile neutropenia (18.8%), nausea (14.6%) and stomatitis (10.4%). Grade II/IV edema was found in 3 patients (6.3%). Combination chemotherapy with weekly docetaxel and doxorubicin is highly active for the treatment of advanced breast cancer with an acceptable toxicity profile.

337 Epirubicin-Docetaxel Administered Simultaneously as First-Line Chemotherapy in Metastatic Breast Cancer.

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The role of taxanes in combination with anthracyclines in first-line therapy of advanced breast cancer is emerging. The objective of this study is to assess the activity of this combination in patients with metastatic breast cancer. 101 patients have been included in the trial up to now. Two patients were male. Mean age of patients was 53 years (range 29-74). Performance status at baseline: ECOG0: 56.4%; ECOG1: 36.6%; ECOG2: 6.9%. The most frequent metastatic site was bone lesions. A total of 480 cycles were administered; mean 4.9 cycles per patient. Dose was reduced for both drugs in 19 cycles. Relative dose intensity both for docetaxel and epirubicin was 0.97. Of the 88 patients who were evaluable for response, 64 reached objective response (72.7%, 95% CI: 62.2-81.7), 29.5% CR, 43.2% PR, 19.3% SD, 7.9% PD. The main toxicity was haematological, with grade 3-4 neutropenia in 10.8% of cycles administered, and febrile neutropenia grade 3-4 in 4.1%. Non haematological toxicity grade 1-2 consisted of nausea (28.9%), asthenia (28.5%) and stomatitis (20.8%). Mean time to progression was 272,5 days and mean overall survival 325.4 days. Our data suggest that docetaxel in combination with epirubicin has major antitumor activity when used as first-line chemotherapy in metastatic breast cancer.

338 Effectiveness of Topotecan as a Primary Chemotherapy of Brain Metastases in Patients with Breast Cancer.

Oberhoff C, Hilfrich J, Kieback DG, Mesrogli M, Sehouli J, von Minckwitz G, von Soest C, Wuerstlein R, Deertz H, Staab HJ, Schindler AE, for the TOPBRAIN-Study Group.

Symptomatic brain metastases occur in approximately 10-15% of patients with breast cancer. Although radiotherapy is recommended as primary therapy, the optimal management remains controversal. Several clinical studies have demonstrated, that primary chemotherapy is effective and improves the overall survival in patients with breast cancer and metastases of the central nervous system (CNS).

Pharmocokinetic studies have demonstrated that topotecan penetrates across the intact blood-brain barrier and that the cerebrospinal fluid penetration exeeds 30-40%. In addition topotecan is effective in advanced breast cancer. Based on this preclinical and clinical data the aim of this pilot-stdy was to evaluate the role of topotecan as a primary chemotherapy in breast cancer patients with CNS metastases.

24 patients (pts) with newly diagnosed, bidimensional measurable CNS metastases received topotecan, 1,5 mg/qm/d, 30-min. infusion for 5 days q 3 week. A total of 93 courses (crs) were administered (range=1-11, median=3 crs/pts). Prior radiotherapy was excluded. Most of the patients had received prior adjuvant or palliative chemotherapy.

8/24 patients were withdrawn from the study for various reasons, 16 pts were evaluable for response: 1 patient had a complete response and 4 had a partial response. 4 pts had stable disease. Hematologic toxicity was the major side effect, non-hematologic toxicity was generally mild and tolerable.

Our results demonstrate that primary chemotherapy with topotecan is an effective and well tolerated treatment in patients with breast cancer and CNS metastases. Based on this pilot-study future clinical protocols should be developed including multimodal treatment strategies (i.e. radiotherapy).

339 Advanced Inflammatory Breast Cancer: Combination Chemotherapy with Doxorubicin and Weekly Docetaxel Treatment.

Quiben R,[1] Méndez M,[1] López P,[1] Enrech S,[2] Menéndez P,[2] Sancho JF,[2] Casinello J,[3] Palomero MI,[4] Perez-Manga G.[4] [1]H. de Móstoles, Madrid; [2]H. Gómez Ulla, Madrid; [3]H. Guadalajara; [4]H. Gregorio Marañón, Madrid, Spain.

The aim of this study was to evaluate the response rate after induction chemotherapy with the combination of doxorubicin and weekly docetaxel. Twenty-seven patients with histopathologically or cytologically proven advanced inflammatory breast adenocarcinoma, with ECOG ≤ 2 and with no previous systemic therapy had been included into the study at the time of this interim analysis. Patients received doxorubicin, 50 mg/m2 iv bolus day 1, followed by docetaxel 36 mg/m2 day 1, 8 and 15. The scheme was repeated every 28 days. The average age of the patients was 55 years, most of them presenting infiltrating ductal carcinoma (66.7%), and had a baseline ECOG=0 (79.2%). The average time from the first diagnosis was 181.1 days (SD=497 days) and the most frequent lesion was in soft tissues (93.9%). The mean dose intensity was 0.83 for docetaxel (SD:3.8), and 0.95 for doxorubicin. All patients had received at least one cycle of treatment, 14 completed treatment and 3 were withdrawn. The total number of cycles administered was 78 with a mean of 2.9 per patient. Response rates were: 33.3% complete response, 57.1% partial response (overall response rate: 90.4%, with 95% CI: [77%, 100%]), 9.5% of stable disease was observed, and no progressions. Grade III/IV neutropenia and febrile neutropenia were reported in 10.3% and 6.4% of cycles respectively. Other grade III/IV adverse events were: nausea (3.8%), asthenia (5.1%), vomiting (1.3%), diarrhoea (1.3%) and stomatitis (1.3%). The scheme administered seems to show a high response rate with acceptable tolerability. Further data on survival and time to progression have to be obtained in order to reach a conclusion about the efficacy of this combination.

340 Weekly Docetaxel in Advanced Anthracycline-Resistant Breast Cancer.

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The aim of the study was to assess the efficacy and safety profile of docetaxel administered in a weekly scheme in patients with advanced breast cancer. 31 patients previously treated with anthracyclines who had progressed on this regimen were included in the study. Nine patients received adjuvant therapy, 9 palliative chemotherapy and 13 both. Mean age was 57.6 years (range 36-75). Baseline performance status was ECOG0: 19.4%, ECOG1: 54.8% and ECOG2: 25.8%. A total of 78 cycles were administered with a mean of 2.5 cycles per patient. In 12 cycles dose reduction was needed and in 25 cycles there was a delay in the administration. Relative dose intensity was 0.97 for patients starting with 36 mg/m2 and 0.93 for patients starting with 40 mg/m2. Complete response was observed in 1 patient (3.4%), partial response in 11 patients (37.9%), resulting in an objective response rate of 41.4% (95%CI: 24-58); 14 patients had stable disease (48.3%) and 3 patients disease progression. Neutropenia grade 3-4 was reported in 19.4% of patients. Other grade 3-4 toxicity were nail disorders (29%), eye disorders (19.4%) and asthenia (12.9%). Weekly docetaxel has significant activity when used as a single cytotoxic agent in patients with advanced breast cancer resistant to anthracyclines.

341 Effects of Adjuvant Anthracyclines on the Activity of Epirubicin Containing First Line Chemotherapy in Metastatic Breast Cancer.

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Published data indicate that a small percentage of patients with metastatic breast cancer who achieve a complete response to first line chemotherapy have a chance to become longterm survivors.Of course the possibility of obtaining a complete response can be increased by the administration of the most active available drugs,which at present are anthracyclines and taxanes. However, the administration of these two agents in the adjuvant setting is becoming of common use. The aim of the present analysis was to evaluate the effect of previous adjuvant anthracyclines on Overall Response Rate, Progression Free and Overall Survival of metastatic breast cancer patients treated with an epirubicin containing first line chemotherapy.This analysis included 312 metastatic breast cancer patients who received a first line epirubicin containing chemotherapy in our institution; None of these patients showed primary resistance to anthracyclines in the adjuvant setting. Of these 182 received FEC (A) and 130 received an association of epirubicin and paclitaxel (B).In the group A 39 out of 182 patients received anthracyclines in the adjuvant setting (25 FEC, 14 Doxorubicin; median age was 54 years (25-72), median PS 0 (0-1), 23% were ER+; dominant metastatic sites were viscera in 44%, bone in 23% an soft tissue in 33%. The ORR was 44%, median PFS 6.6 months and OS 15.8 months.In the group B 27 out of 130 patients received anthracyclines(3 FAC; 1 ABELOFF; 23FEC)in the adjuvant setting; median age was 52 years (range 25-72);median PS 0 (range 0-2), 34% of the patients were ER+; dominant metastatic sites were viscera 64%, bone 30% and soft tissues 6%.The ORR was 85%; the PFS was 12.6 months and OS 24.7 months. Among the 246 patients who did not receive an anthracycline in the adjuvant setting 143 received FEC as first line treatment, with an ORR of 43%,a median DFS of 9 months and a median OS of 15 months) and 103 received an association of epirubicin and paclitaxel with an ORR of 84%, a median PFS of 15 months, and a median OS of 27 months.

Conclusions: Epirubicin is a safe and active drug as first line chemotherapy for MBC. Moreover, due to its favourable cardiotoxic profile it can be safely administered even to patients who received an anthracycline based adjuvant regimen.

342 Schedule-Dependency of Antitumor Activity in Combination Therapy with Capecitabine/Doxifluridine and Docetaxel in Breast Cancer Models.

Tominaga T,[1] Ouchi K,[2] Tanaka Y,[2] and the Docetaxel/Doxifluridine Trial Group.[1] [1]Tokyo, Japan; [2]Oncology, Nippon Roche Research Center, Kamakura, Kanagawa, Japan.

Docetaxel and capecitabine are being prescribed for the treatment of breast cancer. In the present study, we tried to identify the best dosing regimen in combination therapy with these anticancer drugs in human cancer xenograft models. Capecitabine was given p.o. daily for two weeks (day 1-14), whereas docetaxel was given i.v. either on day 1, day 8 or day 15 in a three-week regimen in the MX-1 human breast cancer xenograft. The combination showed a synergistic antitumor efficacy in either dosing regimen. However, the synergistic activity was more obvious when docetaxel was given on day 8. This synergistic efficacy appears to be characteristic of the combination with capecitabine and its intermediate metabolite doxifluridine (5'-dFUrd). Docetaxel given on day 8 also showed potent synergy with 5'-dFUrd, but only slight synergy with 5-FUra or UFT, a fixed combination of tegafur and uracil. Better efficacy was also observed in MAXF401 human breast cancer xenograft and in mouse A755 mammary tumor when docetaxel was given at the middle rather than the other dosing regimen. In contrast, the efficacy in WiDr human colon cancer xenograft was somewhat better when docetaxel was given on day 1. One possible explanation for the synergy is that docetaxel up-regulates tumor levels of thymidine phosphorylase, the enzyme essential for the activation of capecitabine and 5'-dFUrd to 5-FUra. In fact, docetaxel up-regulated the dThdPase levels 4.8 and 1.9 fold in the WiDr and MX-1 models, respectively. However, it did not significantly up-regulate in the MAXF401 and A755 models. Other mechanism, particularly that for the synergy with docetaxel given at the middle during capecitabine/5'-dFUrd administration, would also exist. Based on these observations, a clinical combination phase I/II study with doxifluridine (day 1-14) and docetaxel (day 8) has been initiated in Japan.

343 Reduction of Neurotoxicity from Schedule Modification of Dose-Dense Paclitaxel (P).

Green MC, Buzdar AU, Wingate AD, Hortobagyi GN. Breast Medical Oncology, M.D. Anderson Cancer Center, Houston, TX.

The optimal dose (d) and schedule of paclitaxel (P) remains to be defined. P has schedule-dependent antitumor activity. One study using high-dose weekly (wkly) P (175mg/m^2/week {wk}) resulted in high response rates (86%; Ackerley *et al*; *Semin Oncol*, 1997). In 1998, we initiated a prospective, randomized phase III trial comparing dose-dense P with standard P (225 mg/m^2 every {q} 3 wks) as therapy of operable breast cancer for patients (pts) with positive axillary lymph nodes. Node-positive pts were randomized to either Group I or II. Group I: dose-dense wkly P (175 mg/m^2/wk) for 6 wks followed by a 2 wk break {1 cycle}. Pts received 2 cycles of dose-dense P (12 doses P: dose-density 131.3 mg/m^2/wk). Group II: P 225 mg/m^2 as a 24 hour infusion q. 3 wk for 4 cycles. Both groups received FAC for 4 cycles after completion of P. Of 26 pts who received wkly dose-dense P, 88.5% developed grade 3-4 neurotoxicity. P dose was reduced (150 mg/m^2/wk) with identical scheduling as initially planned {Amendment I: 12 doses P: dose-density 112.5 mg/m^2/wk}. Of 14 pts randomized to the revised dose 50% had grade 3 neuropathy. Even with reduced neurotoxicity, 28.6% of pts did not receive planned therapy due to grade 3-4 neutropenia. In the next cohort of pts, the schedule of wkly P was modified to maintain dose-density while attempting to decrease delays of therapy {Amendment II}. Pts received P (150mg/m^2/wk) for 3 wks followed by a 1 wk break {1 cycle}. Pts received 4 cycles of this therapy (12 doses P: dose-density 112.5mg/m^2/wk). Safety data of the 3 described wkly dose-dense schedules are as follows:

	175 mg/m^2/wk (n=26)	150 mg/m^2/wk Amendment I (n=14)	150 mg/m^2/wk Amendment II (n=7 all D;n=11 First 6 D)
Pts Who Completed All Doses Without Decrease or Delay of Dose	1 (3.8%)	1 (7.1%)	5 (71.4%)
Pts Who Completed 1st 6 Doses Without Decrease or Delay of Dose	9 (34.6%)	6 (42.8%)	10 (90.9%)
Grade 3-4 Neurotoxicity all Doses	23 (88.5%)	7 (50%)	3 (27.2%)
Pts With Delay of Doses Due to Grade 3-4 Neutropenia	7 (26.9%)	4 (28.6%)	1 (9%)

Modification of wkly dose-dense P provides dose-density with decreased grade 3-4 neurotoxicity. The current schedule of 150mg/m^2/wk for 3 weeks followed by a 1 week break {1 cycle} given for 4 cycles maintains dose-density and is comparably well tolerated. This schedule is under continued investigation in this study.

344 Dose-Dense Doxetaxel and Mitoxantrone Is a Promising Chemotherapy in Patients with High Risk Metastatic Breast Cancer.

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Doxetaxel (DCT) and Mitoxantrone (MX) are clinically active and potentially synergistic agents in the treatment of metastatic breast cancer (MBC). This pilot study evaluates the effectiveness and toxicity of the combination of dose-dense (DD) DCT and MX in patients with MBC. 20 patients (52.8 ± 1.9 years) were included in this study. 80% of the pts had visceral metastasis, the average number of metastatic sites was 2.1 ± 0.2. During the first 6 weeks of a 8 week interval, the patients received DCT at 35 mg/m2 q1w and MX at 6 mg/m2 q2w. Patients with regression or steady-state continued the treatment for a maximum of two more intervals. Hematologic parameters were determined on a weekly base to measure the toxicity of the treatment using the NCI criteria. If necessary, pts received granulocyte-colony stimulating factor. During this study 79% (n = 15) benefited with 47% and 32% showing PR and SD respectively. Progressive disease was present in 21% of the pts. 12 pts (60%) developed grade 3 leukopenia. However, no pt was hospitalized due to leukopenic fever or any other life-threatening toxicity. Other hematologic and non-hematologic side effects did not exceed grade 2. This data suggests DD-DCT/MX to be effective in MBC and moderately toxic to the pts. Therefore, we conclude that this regimen is a promising alternative in treating MBC with unfavorable prognosis, which now requires confirmation by large-scaled clinical trials.

345 Dose-Finding Study of UFT Modulated by Leucovorin in Combination with Weekly Paclitaxel in Patients with Metastatic Breast Cancer (MBC).

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Taxol (T) and UFT are agents that acts at different phases of the cell cycle, with non-overlapping patterns of toxicity. Repeated oral dosing of UFT and weekly T would be regarded pharmacologicaly equivalent to continuos intravenous infusion, representing an interesting combination to explore. Based on our previous experience with low toxicity profile with weekly T(Proc. ASCO 1998,740) we decided to determine the maximum tolerated dose(MTD), safety profile and recommended dose of these agents when given concurrently in pts with pretreted MBC. To date, 19 pts: 1M/18 F, median age:56(30-75), PS 0/1: 16, 2: 3 have received 435 weekly doses of therapy at four dose levels(DL). Treatment: consisted on fixed doses of weekly T 80 mg/m2 over 1 hour infusion with escalating doses of UFT (DL I:150, II:200, III:250, IV:300, V:350 mg/m2/day) modulated by leucovorin 90 mg/day, during 21 days followed by 7 rest days. Each cycle represent 28 days. A de-escalated premedication regimen was used. DLT were determined on the basis of toxicity from the first cycle only.

Mild-moderate non-hematologic toxicities (G 1-2) include nausea, fatigue, nail changes and neurotoxicity. Reversible transaminases elevation (G1-2) occurred at DL IV in 4 pts. No hipersencitivity reactions was observed. The longest duration of treatment was 16 months.

Antitumor activity was observed at all DL. UFT plus weekly T appears to be a well-tolerated regimen, with low incidence of myelosuppression and demostrated encorauging activity in MBC pts. Accrual is ongoing to further define the MTD, updated data will be presented. Supported in part by BMS.

The table below summarize the mean ANC nadir and severe toxicities:

DL	(n)	ANC mean nadir	Hem G 3-4	Non-Hem G 3-4	DLT
I	(6)	1828 (1254-2695)	-	1 (diarrhea)	1
II	(3)	1613 (966-2442)	1	-	-
III	(3)	1710 (1102-2405)	-	-	-
IV	(7)	1861 (665-4047)	1	2 (diarrhea)	2

ANC: Absolute neutrophil count

346 Neoadjuvant Chemotherapy (NC) with Cyclophosphamide (CY), Methotrexate (M), 5-FU (F), Prednisone (P), Doxorubicin (A), and Vincristine (V) with G-CSF, Followed by Paclitaxel Plus Cisplatin, (TC) as Adjuvant Chemotherapy in Stage II and III Breast Cancer.

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The objective is to evaluate the efficacy and toxicity of NC with an anthracycline-containing regimen (ACR), followed after surgery by a non-cross resistant regimen with TC. From December 1996 to April 1999, patients (pts) newly diagnosed with stage II and III breast cancer were included into the study. Pts received as NC an ACR every 3 weeks (wks), for a maximum of 6 courses: CY 600 mg/m2/day IV in 1-h infusion day (D) 5, M 40 mg/m2/day IV D5, F 600 mg/m2/day IV D5, P 125 mg/day IV in bolus D1 to D5, A 60 mg/m2 I V in 72-h infusion, and V 2 mg IV D1. Filgrastrim (300mcg) was administered on D7-D17 during NC. Adjuvant chemotherapy, T (175 mg/m2/day IV on 3-h infusion D1) and C (100 mg/m2 IV in 1-h infusion D2) was delivered every 3 wks for 4 courses, after surgery. 10 pts (50%) received hormonal maintenance therapy after TC. 20 pts were analyzed. Median tumor size was 6 cm. Median age was 53 years. IIA 3 pts, IIB 5 pts, IIIA 6 pts and IIIB 6 pts. Median number NC cycles was 6 (range 4-6) and for TP was 4 (range 4-6). Overall response rate after NC was 80% (95% I.C.56.3-94.3) with 2 (10%) pathologic complete response (pCR). Median time to progression (TTP) was 17.4 months. With a median follow-up of 29.9 months, overall survival (OS) was 68%. 115 ACR cycles were delivered. Grade 3-4 neutropenia was seen in 6 episodes (5%), and it was complicated by fever in 5 cases. Grade 3-4 mucositis was noted in 9 cases. 89 cycles of TC were administered with grade 3-4 neutropenia in 9 cases (10%). In our study, the administration of an ACR and TC achieved a favorable TTP and OS despite few (pCR). These two regimens with non-cross resistance could be safely delivered with a tolerable toxicity. Pts recruitment is still ongoing and updated results will be presented.

347 A Multicentric Phase II Trial of Gemcitabine Plus Epirubicin Plus Taxol (GET) as 1st Line Chemotherapy for Metastatic Breast Cancer.

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A single institution phase II trial recently reported, indicated that the GET regimen was feasible, with a good tolerability profile and extremely active (ORR 93% with 31% CR) as Ist line chemotherapy for MBC. The aim of the present study was to confirm these data in a phase II multi-institutional italian trial. Between August 99 and April 00, 39 patients from six institutions entered the study. Treatment schedule was as follows: Gemcitabine 1000 mg/sqm days 1&4 plus Epirubicin 90 mg/sqm day 1 plus Taxol 175 mg/sqm (3 hr) day1 q 3 weeks for a maximum of 8 cycles. Patients characteristics: median age 52 years (range 37-68), median PS 0 (range 0-1); 65% of the patients had positive ER, 69% of them had received a prior adjuvant treatment (with anthracyclines in 17% of the cases); dominant metastatic sites were viscera in 86% and soft tissues in 14% of the patients; 52% of them had at least 2 involved sites of disease. At present all patients are evaluable for toxicity: the good tolerability reported in the previous study seems to be confirmed. In fact G3/4 neutropenia occurred in 59% of the cycles, with 5% of febrile neutropenia, while G3/4 anemia and thrombocytopenia were present in 2 and 7% of the courses respectively. Grade 3 and 4 mucositis was observed in 5 and 3% of the courses respectively . Delays and dose reductions due to toxicity were necessary in 20 and 16% of the courses respectively. 31 patients are now evaluable for response: all of them received at least 2 courses of treatment, 17 received 4 courses and 9 six or more courses. After a median number of 2 administered courses, the overall response rate is 58%, with 10% complete responses, 10% stable disease and 3% progressive disease.In the prior single institutional trial the ORR was 36% after 2 courses, 75% after 4 courses and 92% after 6-8 courses. The final results on the 45 planned patients will be presented.

348 Cardiac Troponin I in Breast Cancer Patients Receiving Anthracycline Chemotherapy.

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Aim: Anthrayclines are among the most effective antineoplastic drugs. They may induce limited cardiomyocyte lysis leading to subclinical myocardial injury. In some, anthracyclines produce overt cardiomyopathy. Increase in blood cardiac troponin level is a sensitive and highly specific indicator of myocardial cell injury. We hypothesised that elevation of cardiac troponin I(cTnI) can identify doxorubicin-induced cardiomyocyte damage at a subclinical level and may be useful as a test for early detection of anthracycline-induced cardiotoxicity.

Matherial and method: CTnI levels were assesed in 11 breast cancer anthracycline-pretreated patients before and 24, 48, 72 hours after succesive course of chemotherapy containing 60 mg/m^2 of doxorubicin. AxSYM Troponin I Microparticle Enzyme Immunoassay was used.

Results: In 2/11 patients (previously treated with anthracycline-containing regimen) TnI level was detectable (0.2 and 0.3 ng/ml) before adriamycin (ADM) administration and increased (0.4 and 11.1 ng/ml) within 72 hours after termination of ADM infusion. In one of these patients ECG revealed transient ischaemia. In both patients there were no echocardiographic signs of cardiotoxicity. cTnI was undetectable before and after ADM infusion in the 9 remaining patients who had no electro- or echocardiographic signs of cardiac damage, either.

Conclusion: Usefulness of cardiac cTnI as an early marker of anthracycline-induced cardiotoxicity needs further investigation, however, may be helpful in identification of patients who require cardioprotection. Updated results will be presented.

349 A Phase II Trial of Escalated Dose Docetaxel (TXT) with G-CSF Support in Patients (pts) with Advanced Breast Cancer.

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We have previously reported a phase I escalation study of TXT, given with G-CSF to reduce neutropenia and sepsis. DLTs were skin and neurosensory toxicity seen in 2/3 pts at the 170mg/m^2 level and 160mg/m^2 was the recommended dose for further study (Proc ASCO 1999, abstract 846). Accrual to a phase II study in advanced breast cancer has been completed. Eligibility criteria were: no previous chemotherapy for advanced disease; PS 0-2; neurosensory toxicity ≤gd I; radiotherapy ≤30% bone marrow; SGOT and SGPT ≤2.5 UNL, ALP ≤5 UNL and bilirubin ≤1 UNL; no CNS metastases. Pts received TXT 160mg/m^{2}q21d with G-CSF (lenograstrim) 5µg/kg/d sc from d2 until neutrophils ≥1.0x10^9/l, and a 3d steroid prophylaxis. 25 pts were entered; currently 20 pts are evaluable for toxicity. Median age was 50 yrs (35-60), 14 pts had previously received adjuvant chemotherapy (prior anthracycline for 11 pts). 50% had liver metastases and PS was 0-13pts, 1-5 pts and 2-2 pts. The median neutrophil nadir occurred on d5 with cessation of G-CSF on d8. Gd IV neutropenia was observed in 10/20 pts (50%). One pt developed febrile neutropenia which was not prolonged. No pts had gd IV anaemia or thrombocytopenia. Other gd ≥III toxicities were asthenia 35% of pts, fluid retention 20%, diarrhoea 15%, nausea 10% and neurosensory 10%. There were no treatment-related deaths. Three pts went off study due to toxicity and 3 pts had dose reductions. Of 14 pts evaluable for efficacy, PRs were seen in 6 pts (43%). Results of mobilisation of haemopoietic progenitor cells will be presented. Docetaxel 160mg/m^2 with G-CSF support may be safely delivered and, so far, does not appear to be more active than the recommended dose of 100 mg/m^2 in metastatic breast cancer patients.≤µ≥

350 Dolastatin-10 in Patients with Advanced Breast Cancer, an NCCTG Study.

Perez EA,[1] Fishkin PA,[2] Hillman DW,[1] Krook JE,[3] Kaur J,[1] Hynes HE,[4] Ingle JN.[1] [1]NCCTG, Mayo Foundation, Rochester, MN; [2]NCCTG, Illinois Oncology Research Association CCOP, Peoria, IL; [3]NCCTG, Duluth CCOP, Duluth, MN; [4]NCCTG, Wichita Community Clinical Oncology Program, Wichita, KS.

This phase II multicenter study evaluated the safety and efficacy of Dolastatin-10 in patients with stage IV breast cancer. Dolastatin-10 is a potent antimitotic agent, derived from the sea hare *Dolabella auricularia*, which binds to tubulin, thus inhibiting microtubule assembly, polymerization of purified tubulin, and GTP hydrolysis. Eligibility criteria included no more than 2 previous chemotherapy regimens (no more than 1 for metastatic disease), measurable disease, and adequate hematopoietic, hepatic, and renal function. Twenty-two patients (pts) were enrolled between 2/99 and 11/99 and 1 patient was deemed ineligible. Patient characteristics included median age = 62 years (range 38-82), performance status 0 (10 pts) or 1 (11 pts), # prior metastatic chemo regimens 0 (11 pts) or 1 (10 pts) and # prior chemo regiments 1 (17 pts) or 2 (4 pts). A total of 57 courses of Dolastatin-10 were administered (median 2, range 1-8) at a dose of 400 mcg/m^2 by intravenous bolus once very 3 weeks. One pt (5%; 95% CI: 0-24%) achieved a PR (partial response) for a duration of 113 days; four pts have had stable disease for a median of 87 days (range 15-121 days). The median time to progression was 45 days. Grade 3 leukopenia developed in 1 pt (2% of courses), grade 3-4 neutropenia in 14 pts (grade 3 - 4 pts, grade 4 - 9 pts, overall - 40% of courses), and grade 3 neutropenic fever in 1 pt (2% of courses). Five pts (19% of courses) received a reduced dose of 300 mcg/m^2; all reductions were due to hematologic toxicity. Nonhematologic toxicities were infrequent and included the following grade 3 toxicities: vomiting (2 pts), diarrhea (1), fatigue (1), nausea (1), speech impairment (1), and confusion (1). Conclusion: Dolastatin-10 in this dose and schedule does not have significant activity in patients with advanced breast cancer. Myelosuppresion is the principal toxicity.

351 A Phase II Study of Oxaliplatin (OXA) and 5-Fluorouracil (FU) in Advanced/Metastatic Breast Carcinoma (ABC) Patients (Pts) Previously Treated with Taxanes (T): Preliminary Results.

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To evaluate efficacy and safety of OXA/FU combination in T ± anthracycline (A) pretreated ABC pts we enrolled pts previously treated with T with at least 6 months (m) since last treatment, progression under prior treatment, no prior OXA or continuous iv FU within 12m, or bolus FU within 6m, and no brain metastases. **Treatment**: OXA 130 mg/m^2/2h followed by FU 1000 mg/m^2/day x 4, q 3 weeks. **Results**: 64 women treated from 10/97 to 10/99 with 57 pts evaluated (March 2000), median age 51 years (34-71). Median nb prior regimens: 2 (1-4). 27 pts resistant to A (AR), 46 to T (TR), and 25 to both (ATR). Median nb involved organs: 2 (1-6). Sites: liver 39 (68%), bone 26 (46%), lung 16 (28%). **Safety**: 57 pts and 277 cycles (cy) available; median nb of cy/pt 6 (1-15). Neutropenia grade (G) 3: 30% (17 pts), G4: 5% (3pts), thrombocytopenia G3: 16% (9pts), G4: 7% (4pts); diarrhea G3: 4% (2pts); nausea G3: 5% (3pts); vomiting G4: 2% (1pt); FU cardiotoxicity 1 pt. Neurotoxicity (specific scale) G3: 3 pts (5%) (after 6 and 9 cy).

	Pts	PR(%)	PD(%)†	SD(%)*	NE
All	57	13(23)	20(35)	19(33)	5(9)
AR	27	7(26)	10(37)	10(37)	3(11)
TR	46	12(26)	16(35)	13(28)	5(11)
ATR	25	7(28)	9(36)	6(24)	3(12)

*≥ 8 weeks, most with ≥ 50% sustained marker decrease, †at/before first evaluation.

All responses were in visceral disease, including 10/39 liver sites (26%). Median PFS 6.9m (4.7-9) **Conclusion:** The OXA/FU regimen shows efficacy and good tolerance in T/A-pretreated/resistant ABC pts, with good disease control rate (PR+SD) and duration. Updated results will be presented.

352 A Phase I Study of Sequential Dose Dense Induction Chemotherapy with High Dose Consolidation for the Treatment of High-Risk Primary Breast Cancer.

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To evaluate the combination of dose density and intensity for patients with high risk breast cancer, we studied a dose dense induction regimen of sequential single agent doxorubicin, paclitaxel and 5-FU followed by high dose chemotherapy (HDC) with autologous stem cell infusion. We treated nineteen patients with Stage IIIB or > 3 lymph node-positive Stage IIB/IIIA breast cancer every 2 weeks X 3 with doxorubicin at 80 mg/m^2, then with paclitaxel at 120 mg/m^2 by 96 hour continuous infusion, and finally with 5-FU by 3-day continuous infusion at either 1655 mg/m^2/day, 1470 mg/m^2/day, or 1285 mg/m^2/day. At the first 5-FU dose level (1655 mg/m^2/day), 37/45 treatments were delivered on schedule. Two of 5 patients developed dose-limiting toxicity (DLT) consisting of hand-foot syndrome and facial edema. The next 8 patients received 5-FU at 1470 mg/m^2/day, with 62/72 courses given on time. Three of the 8 patients developed the DLT of Grade II-III mucositis. Six more patients were treated at a 5-FU dose of 1285 mg/m^2/day; 7 treatment delays resulted in 47/54 courses being given on time. No 5-FU-related DLT was observed in the six patients. Interestingly, grade II-III mucositis was seen with taxol infusion in 12/19 patients, an unusual toxicity most likely related to its administration in close proximity to doxorubicin. Five of the 19 patients enrolled could not proceed to HDC, 1 due to disease relapse, 1 due to insurance denial, and 3 due to the development of cardiac dysfunction (unrelated to 5-FU) on the dose dense regimen. The effect of the triple agent dose dense induction regimen prior to HDC on the CD34$^+$ stem cell harvest, hematologic reconstitution, and toxicity associated with HDC will be reported. We have thus identified 1285 mg/m^2/day every 2 weeks X 3 as the MTD of 5-FU when given as a 72 hour infusion as the third drug in a sequential dose dense regimen with doxorubicin and paclitaxel. However, the excess cardiac toxicity of the overall regimen is unacceptable. It may be feasible to maximize non-cross-resistant drugs by using a different schedule of dose dense therapy.

353 Influence of CD34+ Cell Dose and Tumor Cell Contamination (TCC) of Leukapheresis Products (LP) on Posttransplant Outcome in 76 Patients (Pts) with Metastatic Breast Cancer (MBC).

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We retrospectively analyzed the influence of the stem cell graft on posttransplant outcome in 76 pts with newly diagnosed MBC treated with high-dose chemotherapy (HDCT) between 9/92 and 10/98. Stem cell dose seems to be predictive for outcome after autotransplantation for acute leukemia. In pts with breast cancer, the influence of the number and TCC of infused stem cells is unknown.

Among the 76 pts, a cohort of 25 received double (D)-HDCT after 2 cycles of conventional regimen and 51 received triple (T)-HDCT after one cycle of conventional treatment. At a median follow up of 41.5 months (m) (range, 14 - 88 m) the median progression-free survival (PFS) and median overall survival (OS) of the 76 pts was 13 month (range, 2 - 78 m) and 24.5 months (range, 7 - 78 m), respectively.

A median number of 3.7 x 10^6 CD34+ cells kg^{-1} (range, 0.8 - 38 x 10^6) was administered. In univariate analysis (log-rank test), there was no correlation between the amount of infused CD34+ stem cells and PFS or OS.

One hundred and seven LP collected from 39 pts were assessed for the presence of tumor cells using an immunostaining method with a cocktail of four monoclonal epithelial-specific antibodies. Only 7 of the 39 pts (18%) were transplanted with at least one LP containing tumor cells at a concentration between 0.25 and 2.0/10^6 mononuclear cells. Despite a trend to inferior OS, survival was not statistically different in these pts compared to pts who received an autograft free of tumor cells ($p = 0.54$ for PFS, $p = 0.07$ for OS). Even after reinfusion of tumor cells four of five relapses occurred in old sites of disease, one in the central nervous system.

Our data indicate that there is no influence of CD34+ cell dose on posttransplant outcome in pts with MBC. Regardless of whether occult tumor cells were infused, PFS and OS were not significantly different and the majority of relapses occurred in prior rather than new sites of disease.

354 Dose of Mobilization Chemotherapy Is a Significant Predictor of Outcome for Hormone Receptor Negative Patients Treated with High Dose Cyclophosphamide, Carboplatin and Thiotepa (CTCb) and Peripheral Blood Progenitor Cells (PBPC).

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Between 1994 and 1996, three hundred and eighteen patients with newly diagnosed stage II (4 or more positive nodes) or stage III node positive breast cancer who had received conventional-dose adjuvant chemotherapy were randomized to receive high-dose cyclophosphamide (4 g/m^2), etoposide (600 mg/m^2) and granulocyte-colony stimulating factor (G-CSF) 6 µg/kg/day (HD-Cy, N=156) or cyclophosphamide (2 g/m^2) and the same doses of etoposide and G-CSF (ID-Cy, N=162) followed by the collection of PBPC. 317 of 318 patients had apheresis performed and 316 received CTCb (cyclophosphamide (2 gm/m^2 over 2-3 hours days 1-3), Thiotepa (167 mg/m^2 over 1 hour day 1-3) and carboplatin (267 mg/m^2 over 1 hour day 1-3)) followed by PBPC support. No patient received a taxane during induction therapy. All patients received radiation to the chest wall and hormone receptor positive patients received tamoxifen (10 mg po bid for 5 years) as maintenance therapy. The study was stratified by stage, hormone receptor status and number of positive lymph nodes and analyses for event free survival (EFS) were anticipated a-priori within each of the strata. The median number of CD34+ cells collected following ID-Cy was 19.9 compared to 22.2 x 10^6 cells/kg ($p=0.044$) for HD-Cy. Median follow up for this group of patients is now 50 months. Comparing the two groups overall there was no significant difference in EFS ($p=0.83$). A Cox regression model for EFS identified hormone receptor status and the number of positive nodes as significant predictors. Within the hormone receptor negative group (n=103) the 48 month actuarial estimates of EFS were 65 ± 14% for HD-Cy and 46 ± 15% for ID-Cy ($p=0.06$ log-rank test). The differences in the hormone receptor positive group were not statistically significant ($p=0.13$ log-rank test). We conclude that in hormone receptor negative patients receiving no taxane or maintenance therapy following CTCb, the dose of cyclophosphamide during mobilization may be an important determinant of EFS. Future studies are warranted evaluating better overall treatment strategies for this population.

355 Once-Per-Cycle Pegylated Filgrastim (SD/01) Is as Effective and Safe as Daily Filgrastim in Reducing Chemotherapy-Induced Neutropenia over Multiple Cycles of Therapy.

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We treated 152 breast cancer patients (pts), stages 2 (high risk) - 4, with doxorubicin 60 mg/m^2 and docetaxel 75 mg/m^2 repeated every 21 days for 4 cycles. Prophylactic growth factor support was either sustained duration pegylated Filgrastim (SD/01) or Filgrastim. Pts were randomized to receive SD/01 (100 mg/kg) (n = 47) as a single injection per cycle of chemotherapy or Filgrastim 5 mg/kg injected daily as per package insert (n = 25). The endpoints included duration of severe neutropenia (SN) (ANC < 0.5 x 10^9/L) in cycles 1-4, ANC profile, pharmacokinetics (PK) in cycles 1 and 3, and safety profile of SD/01 in cycles 1-4. The duration of SN in cycle 1 chemotherapy and the SD/01 dose-response were previously reported (Holmes et al, Proceedings of ASCO, 2000; 19:50a. Abstract #191). This abstract reports duration of SN in cycles 2-4. In both groups, mean age was 50 years with most pts (80-90%) being chemotherapy and radiotherapy naive. Although more Filgrastim pts had metastatic disease, this was not predictive of duration of SN in any cycle. The durations of SN in cycles 2-4 ranged between 0-1 days in ≥ 93% of SD/01 pts, compared to ≥ 92% of Filgrastim pts. While SD/01 was administered once per cycle, Filgrastim injections were required for a mean (±SD) of 10.52 ± 2.12 days per cycle. The SD/01 concentration was sustained until the ANC nadir occurred and declined rapidly as the ANC levels started to recover, which is consistent with a self-regulating clearance mechanism. The safety profile of SD/01 was similar to Filgrastim over the 4 cycles, with no significant difference in cytokine-related bone pain. SD/01 at 100 mg/kg administered once per cycle of chemotherapy results in a similar reduction in duration of SN as daily Filgrastim over multiple cycles of chemotherapy.

356 Early Recognition of Taxane-Associated Canaliculitis Important in Preserving Lacrimal Duct Function.

Kneuper Hall R, Metzner-Sadurski JK, Marshall M, Howard GR.

The two available taxanes, paclitaxel and docetaxel, enhance polymerization of tubulin into stable microtubules, interfering with mitosis. Increasing experience using the taxanes at various dosages and schedules has unveiled more subtle side effects, including ocular toxicity. We present six breast cancer patients with ocular toxicity associated with taxane therapy, symptomatically manifested as epiphora (excessive lacrimation). Four patients received docetaxel adjuvantly, and two patients with metastatic disease received docetaxel followed by paclitaxel. Three patients required surgical instillation of temporary or permanent lacrimal duct stents for canaliculitis with lacrimal duct obstruction. Patient 1, after receiving docetaxel for three weeks experienced epiphora, progressive despite close ophthalmologic care, eventually requiring bilateral dacryocystorhinostomy procedures with insertion of permanent Lester Jones (pyrex) tubes. Patients 2 and 3 while undergoing adjuvant docetaxel therapy developed significant epiphora, warranting placement of temporary silicone punctal plugs, 6 months and 1 month post completion of taxane therapy. Patients 4 and 5 developed transient epiphora, eventually resolving without surgery. Patient 6 continues to receive weekly paclitaxel therapy with mild epiphora.

PATIENT	AGENT	SCHEDULE	CUMMULATIVE DOSE AT ONSET OF TOXICITY	INTERVENTION
1	Docetaxel; Paclitaxel	Weekly x 6, Every 3 Weeks x 2;Weekly x 18 months	105 mg/m2 (continuous symptoms on both agents)	Jones tubes 11 months into Taxane Therapy
2	Docetaxel	Every 3 Weeks x 4	100 mg/m2	Temporary Punctal Plugs
3	Docetaxel	Every 3 Weeks x 4	400 mg/m2	Temporary Punctal Plugs
4	Docetaxel	Every 3 Weeks x 4	200 mg/m2	Artificial Tears
5	Docetaxel	Every 3 Weeks x 4	400 mg/m2	
6	Docetaxel; Paclitaxel	Weekly x 4; Weekly x 3	140 mg/m2; 300 mg/m2 (recurrent symptoms)	Artificial Tears

Epiphora that resolves with the cessation of chemotherapy has been identified by investigators using docetaxel; however, permanent stenosis of the punctum and canaliculus has not been reported. Lacrimal gland toxicity may be due to high concentrations of the drug in the tears causing edema of the lacrimal duct epithelium, leading to cicatrical ectropion and dacryostenosis. Patients on taxanes reporting even slight epiphora, should be referred for formal ophthalmologic evaluation. With the increased use of taxanes it is important that medical oncologists be cognizant of this ocular toxicity which may lead to lacrimal canalicular fibrosis with permanent stenosis.

357 Phase I Clinical Trial of Mammary Ductoscopy in Patients with Pathologic Nipple Discharge.

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We have previously shown that endoscopic visualization of human mammary ducts is feasible in mastectomy specimens, particularly in those with nipple discharge. The purpose of this study was to apply mammary ductoscopy to a group of patients presenting with pathologic nipple discharge (PND) prior to ductal excision. Preoperative ductography was attempted in 22/23 patients in order to guide intraoperative ductoscopy. Mammary Ductoscopy was performed on 23 patients with PND using a 1.2mm rigid endoscope with a 350 micron working channel. The patients were given intravenous sedation and underwent a four-quadrant nipple block with local anesthesia. Cannulation of the symptomatic duct was uniformly successful and visualization of the pathologic abnormality was possible in 21/23 patients. Papillary lesions were visualized during ductoscopy and confirmed pathologiclly in 19/24 patients. In one patient, the lesion was seen on ductoscopy but not identified by pathology. Another patient had a focus of DCIS found at the base of the papilloma. In four patients with proximal cutoffs or failed ductogram attempts, ductoscopy revealed normal distal ductal anatomy and assisted the surgeon in a more directed surgical excision. In one patient, an unsuspected papilloma was found deep within the ductal system during ductoscopy. This was not seen on preoperative ductography and likely would have been left behind by standard duct excison. In conclusion, these findings indicate that mammary ductoscopy is feasible and safe in the sedated patient with nipple discharge using a regional nipple block. Ductoscopy may aid in the diagnosis of patients with PND and may also be useful in directing the surgical excision of the pathologic duct.

358 Comparison of Oral Versus Parenteral Administered Bisphosphonates in Breast Cancer Patients with Bone Metastases.

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Introduction: Bisphosphonates are potent inhibitors of osteoclasts. Because of their osteorepair and -protective properties they are established in the treatment of tumor induced osteolysis and subsequent skeletal complications, such as bone pain, pathological fractures and tumor-induced hypercalcemia. Usually the therapy with bisphosphonates is a long-term treatment. Therefore specific side-effects are of great importance. Patients' compliance and therapeutic outcome are of major interest. The primary aim of this study was to compare side effects of parenteral vs. oral regimens and secondly the clinical benefits of the two different drug formulatiions.
Methods: In a randomized, multicenter study from 1995 to 1998 a total of 308 consecutive breast cancer patients with bone metastases were treated. Over a period of 2 years, patients were randomized in three groups and received equipotent doses of either clodronate 2.400mg/die p.o., clodronate 900 mg i.v. (every three weeks) or pamidronate 60 mg i.v. (every three weeks). The bisphosphonates were administered in addition to the usual cytotoxic treatment.
Results: All three groups showed comparatively low levels of adverse effects. Specifically the i.v. clodronate group appeared to be advantageous. As expected, acute phase reactions could only be seen with pamidronate i.v., whereas the most frequent gastro-intestinal side effects were detected with oral clodronate n=11 (6,41%). Fractures were observed in 9 patients in the i.v. clodronate group, 14 in the oral clodronate group and 9 in the i.v. pamidronate group. Most effectively pain was reduced by oral clodronate (89%), followed by pamidronate i.v. (80%) and intravenous clodronate (72%).
Discussion: The results of this study show that the therapy with bisphosphonates is well tolerated. I.v.-administered clodronate shows a comparatively lower side-effect profil with similar clinical efficacy compared to pamidronate. The most effective pain reduction was achieved with oral clodronate, whereas no differences could be shown in the reduction of pathological fractures.

359 Prinomastat Inhibits Primary Tumor Growth and Retards Osteolytic Disease in Xenograft Models of Breast Cancer Metastasis.

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Prinomastat (AG3340) is a potent metalloproteinase (MMP) inhibitor with pM affinities for gelatinases (MMP-2 and -9) as well as MT-MMP-1 (MMP-14) and MMP-13. It has demonstrated a broad spectrum of antitumor activity in a variety of rodent tumor models and is currently undergoing clinical trials in lung, esophageal and prostate cancer protocols. In the present study, we have used two *in vivo* (xenograft) models of breast cancer which make use of the MDA-MB-231 (human) breast cell line transduced with a retroviral tag (Bgal) to allow quantitation of tumor burden. In the first model, 231-Bgal cells were inoculated orthotopically into the mammary fat pad of nude (Balb/c) mice, and drug dosing commenced twice daily (PO) with either vehicle, 25, or 50 mg/kg/dose (n=8/group) when tumors became palpable. A significant suppression of primary tumor growth was observed in a dose dependent fashion, reaching statistical significance by day 35 ($p < 0.05$). By day 60, average tumor volumes for the 25 and 50 mg/kg/dose groups were 59% and 70% smaller, respectively, by comparison to the vehicle control group. The other model specifically mimics breast-to-bone metastasis and entails intracardiac (left ventricle) inoculation of the 231-Bgal cell line. In this series, either vehicle control or prinomastat dosing (50 mg/kg, PO, twice daily) commenced 48 hrs prior to breast cell inoculation and continued for the duration of the experiment. Osteolytic bone damage was monitored by periodic X-ray analysis of anesthetized mice and was notably retarded for the drug treated group: by week three, 3 of 10 mice had damaged limbs compared to 8 of 10 for the control (no drug) group. Endpoint analysis of soft organ metastasis by TaqMan PCR is ongoing. Prinomastat was well tolerated by mice at the doses indicated. These data demonstrate the efficacy of prinomastat in an orthotopic human mammary tumor xenograft setting, and show for the first time the ability of this class of agent to reduce osteolytic disease associated with bone metastasis.

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360 A Phase II Study with Lutrin® for Recurrent Cutaneous Breast Cancer: A Redox Active Photosensitizer with a Dual Mechanism of Action That Potentiates Response to PDT and Chemotherapy.

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Motexafin lutetium (Lu-Tex, Lutrin®) is currently being studied in a Phase II clinical trial as a photosensitizer for the photodynamic treatment (PDT) of cutaneous breast cancer metastases in patients previously treated with radiation. Lu-Tex is an expanded porphyrin molecule, which when activated by 732nm light produces singlet oxygen. Phase I and II studies have demonstrated some tumor selective localization and rapid clearance from the plasma. Lutrin is electron affinic with a reduction potential of -44 mV (NHE). Based on its electrochemical properties, we evaluated the reaction with intracellular reducing metabolites in the absence of light. Lu-Tex was found to catalyze the oxidation of NAD(P)H and ascorbate under physiological conditions, leading to hydrogen peroxide formation. Catalytic rate constants k_{cat} and K_M were found to be 0.12 ± 0.01 min^{-1} and 563 ± 200 μM for NADPH and 0.038 ± 0.0009 min^{-1} and 49 ± 7 μM for ascorbate oxidation, respectively. Human uterine cancer cell proliferation was inhibited in vitro by Lu-Tex and ascorbate or NADPH. This effect was abrogated by the addition of catalase or superoxide dismutase. Moreover, the fluorescence of the dye DCFA was enhanced in cells pre-treated with Lu-Tex, indicating reactive oxygen species generation without light exposure. In the ongoing Phase II trial, patients who have received multiple prior chemotherapy regimens without effect, have shown tumor regression after the combination of Lu-Tex PDT and systemic chemotherapy. Clinically significant reductions in treated tumors have been seen in 4 out of 5 patients. Lu-Tex administration is well-tolerated and no significant increase in toxicity has been reported with combined systemic therapy and Lu-Tex PDT. These data lead us to suggest that Lu-Tex's redox activity may contribute to potentiation of its photodynamic effect and possible synergism with chemotherapeutic treatments in breast cancer patients.

361 Use of Bisphosphonates in Breast Cancer Metastatic to Bone at a Regional Cancer Centre.

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Objective: To study the effect of the publication of evidence-based recommendations on the prescribing pattern of bisphosphonates to women with breast cancer metastatic to bone.

Methods: We conducted an audit of 1682 consecutive ambulatory clinic patients seen by five Hamilton Regional Cancer Centre (HRCC) medical oncologists specializing in breast cancer to determine the number of patients eligible for bisphosphonate therapy as outlined by the Ontario Cancer Treatment Practice Guidelines Initiative Evidence-Based Recommendation Report. We reviewed those patients with bone metastases seen in clinic from January 1, 1997 to August 30, 1997 and from September 1, 1997 to November 30, 1998. The proportion of eligible patients receiving bisphosphonates was determined for each medical oncologist, including whether bisphosphonates were initiated within two months of diagnosis of skeletal metastases. Documentation of reasons for non-treatment were compared to physician response on a follow-up questionnaire.

Results: Thirty patients of an eligible 80 (37.5%, range 16.7-47.6%) from January to August 1997 and 18 of 46 eligible patients (39.1%, range 0-100%) from September 1997 to November 1998 received bisphosphonates. A significant increase in the number of patients receiving bisphosphonates within two months of diagnosis of skeletal metastases was evident in the post-recommendation period (Sept 97-Nov 98). There were also fewer patients waiting more than two months after diagnosis of skeletal metastases to receive bisphosphonates during the later time period (p=0.0001). The most important reason for non-initiation of therapy was fear of complicating a patient's management with too many medications. Other reasons included a perceived unfavourable side-effect profile of oral bisphosphonates, controversy regarding asymptomatic or single bone metastases and conflicting with protocol therapy in patients on clinical trials.

Conclusion: Despite published literature and evidence-based recommendations to support their use, bisphosphonates were used in less than 40% of eligible patients at the HRCC. A statistically significant trend toward earlier prescribing of bisphosphonates following the publication of recommendations was seen. The wide range in the use of bisphosphonates suggests individual oncologists interpret the evidence very differently.

362 Primary Lymphoma of the Breast: A Retrospective Analysis of 11 Cases.

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Primary lymphoma of the breast are rare and represent less than 0.6% of all mammary malignancies. However, histopathological features, therapeutic approach and outcome are still matter of debate. The mammographic appearance is unspecific and so the final diagnosis can usually not be made before examination of paraffin-embedded tissue.

Results: A retrospective analysis of 11 primary lymphoma of the breast (PLB) was undertaken. The patient population consisted of 11 women with a mean patient age of 64.8 years (range 33 to 79y). All the tumors presented with features of breast carcinoma. At time of diagnosis all patients had unilateral involvement with predominant right breast lesions (9)/left (2). Surgical procedures included wide excision biopsy in seven patients and modified radical mastectomy in four. We identified 10 cases of B-cell lymphomas and 1 T-cell-lymphoma. 10 patients received systemic chemotherapy and/or radiation and one received only locoregional radiotherapy. The follow-up period ranged from 3 to 78 months (mean 22.1m.). The OS and RFS at mean were 81.8% and 72.7%.

Conclusion: PLB behave like lymphomas of similar histological types and stages presenting at other sites. Frozen-section histology is unreliable for diagnosing lymphoma of the breast. There is no survival advantage for patients who underwent radical surgery.

363 WITHDRAWN

364 Breast Conserving Therapy (BCT) of Ductal Carcinoma In Situ (DCIS): Size of the Lesion Is the Main Risk Factor for Residual Tumor.

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The complete excision of DCIS as BCT should cure this non-invasive non-metastazing lesion definitely. In contrast to mastectomy (ME) in BCT is an increased probability (up to 40%) of local recurrence caused by residual tumor. We examined the impact of extension, margin status, and grading of DCIS on the estimation of residual tumor risk. 217 (19.7%) out of 1096 breast cancers operated in women during a 5 year period in our Breast Unit were DCIS. In 205 of them an excision was performed to attempt BCT. The specimens were examined according to the "Berlin-Buch practice protocol" developed by our multidisciplinary group in 1992. The pathological examination based on a standardised sampling with consideration of the ductal orientation for estimating the diameter of the DCIS by combination of direct measuring and reconstruction. In every case with DCIS detected in paraffin slides within a distance of 5 mm to the resection lines (RL) a re-excision (RE) followed. Free margins of more than 10 mm were found in 60 of the 205 patients; in the remaining 145 cases DCIS was detected within 5 mm to the RL and a RE has been performed. REs were necessary only in DCIS larger than 16 mm. All RE specimens with tumor-free margins of 5 mm at the same time showed free margins of 10 mm. In all 136 DCIS larger than 40 mm this could not be reached.

Size of DCIS (mm)	< 15	16 - 40	>40
n =	46	23	136
Primary Excision with Free Margins (5mm)	46 (100%)	14 (60.9%)	0 (0%)
Definitive BCT with Free Margins (10mm)	46 (100%)	23 (100%)	0 (0%)

Therefore ME was recommended to them. In the ME specimens an involvement of at least one additional quadrant and/or the central gland has been proved. There was no significant statistical relationship between the grade of differentiation and the size of DCIS. Standardised pathological examination with microscopic evaluation of the margins of excisional specimens is the only way to define adequacy of resection and the extent of DCIS. The extension of a DCIS area over 40mm in diameter means a high risk of involvement of more than one quadrant of the breast and represents a contraindication for BCT.

365 Rural-Urban Differences in Radiation Therapy for Ductal Carcinoma In Situ (DCIS) of the Breast.

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Rural women in the US are at a documented disadvantage with regard to breast cancer detection, diagnosis, and treatment and generally do not receive state-of-the-art therapy. The objective of the study was to determine if, and to what extent, rural women were less likely to receive radiation therapy (RT) following breast-conserving surgery (BCS) for DCIS, the recommended treatment for DCIS.

Our analyses were based on 1991-1996 data provided by the Surveillance, Epidemiology, and End Results (SEER) Program and the Area Resource File for the availability of RT facilities in each county. BCS, with or without RT, was defined according to the SEER definition of site-specific surgery for breast cancer. Women were considered rural if they did not reside in a Metropolitan Statistical Area at the time of diagnosis. Multiple logistic regression was used in the analysis.

During this time period, 6,884 women were treated with BCS for DCIS, 50.9% of whom received RT. Rural women were less likely to receive RT (OR=0.38) as were women who resided in counties without RT facilities (OR=0.77). Differences in factors associated with receipt of RT between younger (<65) and older (65+) women were found. Younger rural women were less likely to receive RT than their urban counterparts (OR=0.38), while no difference was found among older women. Local availability of RT was not associated with receipt among younger women, while older women without this availability were less likely to receive RT (OR=0.48).

In conclusion, barriers to RT following BCS for DCIS may be different between younger and older rural women relative to their urban counterparts.

366 Avoiding Radiotherapy Following Breast Conserving Surgery for Ductal Carcinoma In Situ of the Female Breast.

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The role of radiotherapy (RXT) in the management of ductal carcinoma in situ (DCIS) remains controversial when wide surgical resection margins can be assured. There are considerable patient benefits to be gained together with economical savings by avoiding RXT. The management of DCIS in our institution is based on segmental resection (quadrantectomy) and further local resection for positive margins, aiming to remove the DCIS together with the area at risk of recurrence. If margins cannot be cleared by local surgery or multifocal disease is present as defined mammographically and on magnetic resonant imaging (MRI), mastectomy with immediate reconstruction is advised. All patients receive annual mammographic surveillance.

Complete medical records, pathological reports and follow up details were reviewed for 125 women with DCIS treated since 1982. Amongst these patients, 62 were treated by breast conserving surgery alone (BCS) (Group 1), 12 by BCS and adjuvant RXT (Group 2) and 51 had total mastectomy (Group 3).

In Group 1, the median age was 57 years (range 38-87). To ensure adequate resection margins, 25 patients required further excision following BCS and residual disease was found in 32%. There were 50% high grade tumours, comedo necrosis in 26% and microinvasion in 14.5%. At a median follow up of 45 months (range 1-150), local recurrence occurred in 1 patient (1.6%) who originally had high grade DCIS with microinvasion. In Group 2, the median age was 54 years (range 35-61). All patients had high grade DCIS, 7 with microinvasion and 2 had comedo necrosis. At a median follow up of 63 months (range 3-134), there were 3 local recurrences (25%): 2 invasive ductal carcinoma, (1 nodal metastasis) and 1 patient had 2 recurrences at 5 years of DCIS and 11 years of invasive carcinoma. In Group 3, the median age was 56 years (range 31-79), 23 patients received primary mastectomy for radiologically obvious multifocal disease and 28 patients had mastectomy for positive margins following BCS. Residual disease was found in 62.9% of further resected specimens. At a median follow up time of 54 months (range 1-144), 1 patient has developed ipsilateral nodal metastasis.

It is possible to select a group of patients with DCIS who may be treated with BCS without RXT. Our early results based on very wide local resection question the validity of existing prognostic indices for this disease. Further follow up is planned to substantiate these findings.

367 Dilemmas in the Management of DCIS: A Survey of Patterns of Care in the United Kingdom.

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Although widespread implementation of evidence-based guidelines has impacted on the outcomes for invasive breast cancer, it is not clear that clinicians are using the results of recently published randomised controlled studies to aid decision-making in their management of DCIS.The aim of this study was to document current views of clinicians on optimal management of DCIS of the breast with regard to extent of surgery, and use of adjuvant therapies(radiotherapy and tamoxifen)

Methodology:A postal questionnaire was sent to 270 breast surgeons referred patients from the UK breast screening centres and 203 clinical oncologists treating breast cancer at each UK Cancer Centre.

Results:The overall response rate was 71%. There were significant workload variations with 61% of surgeons managing 2 or less cases per month; 84% of clinical oncologists saw 2 or less cases postoperatively to discuss adjuvant therapies. There was general agreement on the need for clear resection margins, with 97% of respondents recommending re-excision for positive margins. Mastectomy was felt to be optimal management by 80% based on size of lesion, or quadrantic extent. 54% of surgeons considered some form of axillary surgery to be indicated (42% of these if mastectomy undertaken;28% for cases with microinvasion;15% for high grade lesions; 14% for large or multifocal disease; 3% all cases).Use of adjuvant radiotherapy(RT) varied markedly between respondents. Equal numbers (14%) offered RT to all cases after lumpectomy or none!Wide variations were noted in perceived indications for adjuvant tamoxifen. 26% considered for all patients, and 21% never used. The largest single indication for use was presence of microinvasion (33%). 27% used ER results to guide usage. 15% considered a positive family history to indicate a role for tamoxifen.Conclusions:Although DCIS now comprises 20-30% of all screen -detected breast cancer, a large number of clinicians in the UK treating breast cancer manage relatively few cases per annum. The perceived indications for all 3 major treatment modalities (mastectomy vs BCS, radiotherapy, tamoxifen) were noted to vary widely, reflecting a) continuing uncertainty of the evidence-base for the management of DCIS; b) the need for consensus guidelines based on recently published randomised studies.

368 Using a Risk-For-Distress Measure to Predict Risk for Treatment Refusal.

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The Omega Screening Questionnaire (OSQ) is an instrument designed to predict risk for later emotional distress in patients beginning cancer treatment. The risk-for-distress score is based on 20 predictors, including demographic, socioeconomic, and social information; physical and mental health history; current symptom distress; and current concerns. In previous studies by Mood et al, breast cancer patients with scores ≥7 were found to have higher emotional distress and lower quality of life scores up to 6 months post-treatment. In the current study, the OSQ was distributed on an administrative basis to all new cases of invasive breast cancer to identify the problems patients attending KCI were confronting. It soon became evident that patients, especially African-Americans (AA), with high risk scores had a significantly higher rate of refusal of recommended therapy. Given the reduced survival rates from breast cancer in the AA compared to the Caucasian (CC) population, and the renewed national effort (ASCO, 2000) to ensure adequate cancer treatment for all, we pursued this finding. In the first 224 recommendations for treatment, of patients with OSQ scores <7 who were offered adjuvant radiation, 3 of 36 refused; for adjuvant chemotherapy, 6 of 68. For patients with scores ≥7, the refusal rates were 8/28 and 9/42 respectively ($p<.01$). Refusal rates for tamoxifen were not related to OSQ score. The refusal rate for AA's (34.3%) was more than twice that of CC (13.1%)($p<.02$). Refusal rates for therapy recommendations were not correlated with Brief Symptom Inventory (BSI) scores, a measure of acute distress at the time of diagnosis, even though BSI and OSQ scores did correlate with each other, ($r=.61$ $p=.0001$). The preliminary results from this unique inception cohort study suggest it is possible to detect potentially non-compliant patients (PNCP) before the fact. Scores on the OSQ might form the basis for structured pre-emptive interventions to improve compliance if its sensitivity (65%) and particularly its specificity (24%) for detecting PNCP could be improved. For that purpose, further analyses, including subscore examination, are currently underway.

369 Rapid Bone Loss after Chemotherapy in Breast Cancer Patients.

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We studied the influence of standard adjuvant chemotherapy on the bone mineral density (BMD) of 27 women (mean age: 49,29 years) diagnosed of breast cancer with I-III stage. BMD (g/cm2) of the lumbar spine and proximal femur (femoral neck, trochanter, intertrochantereal area and Ward's triangle) was measured by dual-energy x-ray absorptiometry (DEXA) before and after adjuvant chemotherapy. We also evaluated body mass index (BMI), hormonal history, hormonal levels and biochemical markers of bone turnover. 16 women were premenopausal and 5 of them became amenorrheal after chemotherapy.

After treatment, women showed a general signifficant loss of bone at lumbar spine (1.032±0.14 →1.009±0.15; p=0.001), trochanter (0.703±0.12→ 0.686±0.11; p=0.007), and total proximal femur (0.926±0.14 → 0.911±0.13; p=0.018). There was an increase of plasma luteinizing hormone (LH) (mUI/ml) (12.1 ±9 →32.4±19; p< 0.0001) and follicle-stimulating hormone (FSH) (mUI/ml) (27.1±27 → 64.5±33; p <0.0001) as expression of induced hipogonadism. Bone turnover markers also increased after treatment: seric osteocalcin (ng/ml) (16.5±7→ 21.7±9; p=0.03) and urinary pyridinolines (nM/ml) (10.2±3→ 12.4±2; p=0.039).

Our patients showed a rapid bone loss and a increased of turnover markers after adjuvant chemotherapy. The 4-6 months between first and second densitometry do not explain the bone loss. This could be explained by chemotherapy effect or by the induced hipogonadism. Another cause could be the effect of the tumor itself, but none of the 27 patients had a relapse during the study period.

370 First Report of a Comprehensive Survey of Chronic Arm Morbidity after Curative Breast Cancer Treatment - Incidence and Its Impact on Quality of Life.

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Objective: To study chronic arm morbidity after curative treatment for breast cancer, including its incidence, the relative contributions of surgery & radiotherapy and its impact on quality of life (QoL).

Methods: A simple 7 question screening survey was developed and mailed to a random sample of 744 breast cancer patients referred to two cancer centres, which serve as the only tertiary referral centres for a catchment population of 2 million. These patients were treated in the years 1993-1997. There was a minimum of 2 years between diagnosis and survey. All had curative treatment with no evidence of recurrence. Patients had a various combination of treatments to the axillae (radiation & axillary dissection) which will be discussed at presentation. Respondents were classified as with (group 1) or without (group 2) arm problems based on the survey. Random samples of each group were then invited for a detailed assessment of their symptoms and physical signs. Their QoL was assessed by the validated EORTC QLQ-C30 questionnaire as well as a detailed arm problem questionnaire looking into arm function, pain, stiffness and treatment.

Results: The following preliminary results are available at manuscript preparation: 63% (467/744) of the surveys were returned. Validation analyses showed that the screening survey was an excellent tool in identifying the two components of arm morbidity: objective arm edema & subjective arm discomfort. 46.7% of patients were classified as group 1 (with arm problems) based on the screening questionnaire. 17.5% of group 1 Vs 1.8% of group 2 patients had an arm volume difference of >200cc. Further analyses are being done to further characterize the two groups of patients, as well as the relationships of arm morbidity to treatment factors (surgery & radiotherapy) and disease factors (number of nodes, extranodal extension).

Conclusions: Data collection was complete and the initial survey was validated to be a good screening tool. Ongoing analyses are being done to look into the incidence of the various components of arm morbidity, their impact on the QoL of patients as well as the contributing factors for their development. These will be presented at the meeting.

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371 Metastases to the Breast: Determinants of Survival.

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Metastasis (mets) to the breast from non-breast malignancies represents an uncommon, but challenging clinical scenario. The purpose of this study was to determine the factors that impact survival in this patient population.

Methods: Between 1983 and 1998, 260 patients (pts) with mets to the breast were treated at a comprehensive cancer center. Their records were reviewed and survival determined by the Kaplan-Meier method.

Results: Median age was 51 years (range,13 to 85). 229 patients (88%) had a prior history of cancer. At presentation 58 pts (22%) had no other evidence of disease and 202 (78%) had active and/or metastatic disease. Of the 260 pts, 158 (61%) presented with a solitary mass and 218 pts (84%) had unilateral disease. Pathological diagnosis was obtained in 213 pts (82%); primary malignancies included melanoma in 79 (30%), lung in 37 (14%), unknown primary in 29 (11%), ovary in 23 (9%) and gastrointestinal in 11 (4%) and 34 (13%) other. Upon review, 13 pts initially referred with a pathological diagnosis of breast cancer were later diagnosed with nonbreast metastatic disease at our institution. On initial detection of a breast lesion 102 pts were surgically treated (98 with wide local excision, 4 with mastectomy); 31 (30%) of these developed a local-regional breast recurrence. Of the 158 pts who did not have an initial procedure, 17 (11%) had a delayed procedure: 3 for local complications, 6 for tumor vaccine/chemosensitivity, 8 for other reasons. Median disease-specific survival (DSS) for patients with breast metastases was 10 months(m). Median DSS was shorter in pts with a previous cancer history (10 v 15 m, P=0.03), active/ other metastatic disease at presentation (8 v 29m, P<0.001), pathological confirmation of mets (6 v 11m, P<0.001), multiple lesions (8 v 10m, P=0.03), bilateral mets (7 v 11m, P=0.04), and non-neuroendocrine histology (P=0.006).

Conclusions: Metastasis to the breast, though not common, must be considered in the differential diagnosis of a breast mass, especially in patients with prior cancer history. Expected survival is poor; local therapy should be tailored to the individual patient.

372 CD34, CD117 and Actin Expression in Phylloides Tumor of Breast.

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Introduction: Many reports suggest that phylloides tumors (PTs) are neoplasms of stromal cells. The stromal cellularity, cellular atypia and mitotic activity of PTs were used as the indicators to differentiate benign from malignant PTs. This study correlates the expressions of CD34, CD117 and actin in the benign and malignant phylloides tumors.

Materials and methods: Using immunohistochemical analysis, we investigated CD34 and CD117 expression in 19 cases of PTs (7 benign, 12 malignant). Additionally, actin (HHF-35) expression was also studied because actin-positive cells were usually absent in normal mammary stroma. Mutation screening within the juxtamembrane region of CD117 was also performed.

Results: 6 of 7 benign PT stromal lesions stained positively for CD34, while only 3 of 12 cases of malignant PT were focally CD34 positive (P = 0.0106). CD117 expression is associated with malignant potential of PTs (P=0.0106). Actin expression was found in 8 of 12 cases of malignant PTs, but in only 1 of 7 of benign PTs (p=0.027). Actin expression was significantly (P=0.04) correlated to frequent mitotic activity (> 5 mitoses per 10 high-power fields).

Conclusions: The expression of CD34 was associated with benign PTs, while CD117 and actin were preferentially expressed in malignant PTs. These immunohistological markers might be useful for the histopathological grading of PTs.

373 Overexpression of HIF-1α in Breast Carcinogenesis.

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Hypoxia Inducible Factor 1 (HIF-1) is a transcription factor thought to play an important role in tumor growth and metastasis by regulating cell metabolism and inducing angiogenesis in order to survive cellular hypoxia. Overexpression of the HIF-1α subunit in different human cancers has already been demonstrated. The expression of HIF-1α at different stages of breast carcinogenesis was determined and correlated with VEGF, HER-2-neu, p53, Ki-67, estrogen receptor (ER) status and microvessel patterns as possible target biomarkers.

Immunohistochemistry using monoclonal antibodies against HIF-1α, VEGF, HER-2-neu, p53, Ki-67, CD31 and ER was performed on paraffin embedded sections including the six major diagnostic categories of breast lesions.

Neither normal breast tissue (N=10) nor ductal hyperplastic lesions (N=10) showed HIF-1α overexpression. Overexpression was found in 4/10 well differentiated ductal carcinomas in situ (DCIS) and 6/10 well differentiated invasive breast cancers. Also the poorly differentiated cases showed an increase in HIF-1α overexpression from DCIS (8/10) to invasive cancer (10/10). HIF-1α overexpression was positively correlated with Ki-67 ($p=0.002$), VEGF ($p=0.002$) and ER positivity ($p<0.0001$). HER-2-neu, p53 and microvessel density were not significantly correlated although a positive trend with HIF-1α overexpression was seen. VEGF expression showed a nearly significant correlation with the microvessel density ($p=0.067$).

Conclusive, HIF-1α overexpression occurs in DCIS and invasive breast cancer, especially in the poorly differentiated lesions. These data indicate that HIF-1 plays a role in breast carcinogenesis in vivo. Moreover, HIF-1α overexpression is associated with increased proliferation and poor differentiation grade in breast lesions, suggesting a correlation between HIF-1 activity and tumor aggressiveness. The positive correlation between HIF-1α expression and the ER status provides a new perspective on the role of HIF-1 in breast cancer. Altogether, these data support the hypothesis that progressive increases in HIF-1α expression contribute to the process of breast tumor progression.

374 Hydroxymethylacylfulvene (HMAF, MGI 114, Irofulven) Induces Apoptosis in the Caspase-3 Deficient Breast Cancer Cell MCF-7.

Herzig MCS, Liang H, Woynarowska B, Woynarowski JM.

Breast cancer cells are often refractory to drug induced apoptosis, presumably due to a lack of a key executioner component of an apoptotic pathway, caspase 3. HMAF is a novel agent with alkylating properties, currently in Phase II trials, with the potential to differentiate between tumor and normal cells in apoptosis induction. This study explores the potential of HMAF to circumvent caspase-3 deficiency and promote apoptosis in breast cancer cells. We characterized apoptotic responses to HMAF in the breast cancer cell line MCF-7 which does not express caspase 3 and the normal human mammary epithelial cells (HMEC), which are caspase-3 positive. Our results confirm that HMAF is a potent inducer of apoptosis in MCF-7 cells despite their lack of caspase-3. Significant, time-dependent apoptosis was detected in the MCF-7 cell line as measured by fragmentation of radiolabeled DNA. For example, after 12, 24 and 48 h incubation with HMAF at 1μM (~5 X IC50), fragmented DNA comprised 3.7, 20.2 and 55.4% of total DNA, respectively. Normal HMEC cells were refractory to 1 μM HMAF with ~3-9 % fragmented DNA after 12-48 h, although they showed significant apoptosis after 24-48 h exposures at drug levels > 3 μM. Thus, HMAF shows some, but limited, preference for cancer breast cells. The broad spectrum caspase inhibitor Z-VAD-fmk inhibited HMAF induced apoptosis of MCF-7 cells in a concentration dependent manner. e.g., 2-200 μM Z-VAD-fmk decreased HMAF-induced apoptosis by 10-85 %, respectively. Caspase processing and catalytic activity in HMAF-treated MCF-7 cells were also analyzed. The results show that whereas the caspase cascade does mediate HMAF-induced apoptosis, caspase-3 expression is not critical for HMAF apoptotic potential. MDA-MB-231, a breast cancer cell line that is caspase-3 proficient, was somewhat less apoptotic in response to HMAF than MCF-7 cells. The results point to the potential utility of HMAF as a pro-apoptotic modality against caspase-3 deficient breast cancers.

375 CP461 Induces Apoptosis and Growth Inhibition of Breast Cancer Cells Independent of HER2/neu Receptor Expression.

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CP-461 is one of a new class of pro-apoptotic drugs termed selective apoptotic anti-neoplastic drugs (SAANDs) that has been shown to enhance the anti-tumor effects of taxane chemotherapy in MX-1 mammary tumor xenografts. The drug induces apoptosis in a wide range of human cancer cells and like other SAANDs lacks cyclooxygenase inhibition. CP-461, a potent derivative of exisulind, was selected as a higher affinity cGMP phosphodiesterase inhibitor that activates cGMP-dependent protein kinase to induce apoptosis. The objective of this study was to evaluate the growth inhibitory effects of CP-461 alone or in combination with Herceptin or Taxotere on human breast tumor cell lines (MCF-7, MDA-MB-231, MDA-MB-435S, MDA-MB-436, BT-20) with low HER2/neu receptors and cell lines (BT-474, SR-BR-3, MDA-MB-453) over-expressing this growth factor receptor. CP-461 inhibited all eight breast cell lines with nearly 100% effect with an IC_{50} = 0.5-0.9 μM which was not dependent on HER-2/neu and estrogen-receptor expression. The EC_{50} for apoptosis (0.8 μM) was the same in MDA-MB-453 over-expressing HER-2/neu receptors and MCF-7 cells, lacking over-expression. When growth inhibition was studied in BT-474 with multiple drug effect/combination index (CI) isobologram analysis using constant molar ratio dilutions of Herceptin/CP-461, a CI < 1 was found indicating a synergistic effect. MDA-MB-435S cells, lacking Herceptin receptors, showed no effect of Herceptin on activity of CP-461. Additionally, CP-461/Taxotere combinations showed synergism in BT-474 cells. These data suggest that like exisulind, SAANDs such as CP-461, merit further testing in breast cancer, either as a single agent, or in combination with Herceptin and/or taxanes.

376 Treatment with the Pure Antiestrogen Faslodex (ICI 182780) Increases the Sensitivity of MCF-7 Breast Cancer Cells Against Fas Induced Apoptosis.

Diel P, Loeffek S, Smolnikar K, Michna H. Morphology and Tumor Research, DSHS, Cologne, Germany.

Recently we have demonstrated that pure antiestrogens are able to induce active cell death in human breast cancer cell lines. There is evidence that beside members of the Bcl-2 family the TNFR1/Fas signal transduction pathway is involved in the molecular mechanisms of apoptosis induction by antiestrogens. We have analyzed the time dependent expression of members of the Bcl-2 family (Bcl-2, Bax) and members of the Fas associated signal transduction pathway (Fas, FasL) during apoptosis induction by the pure antiestrogen ICI 182780 (ICI) in MCF-7 cells. Cells were cultured in the presence of 10-10 M Estradiol (E2). Coadministration of ICI [10-7 M] leads to a significant and time depended decrease of Bcl-2 mRNA and protein expression. In contrast Fas mRNA and protein expression were significantly induced by antihormone treatment. To analyze if the induction of Fas-expression is functionally involved in the growth inhibitory action of the pure antiestrogen, MCF-7 cells, cultured in the presence of 10-10 M estradiol (E2) and ICI [10-7], were treated with Fas activating antibodies. A significant increase of Fas induced apoptosis was observed during antihormone co-treatment. Our results demonstrate that treatment with antiestrogens leads to a higher sensitivity of MCF-7 cells against Fas induced apoptosis. We conclude that the Fas signaling pathway is functionally involved in apoptosis induction by antihormones and may be an interesting target for the analysis of the development of antiestrogen resistance of tumors of the breast.

377 Measurement of Apoptosis in Breast Cancer by Immunohistochemistry Using an Antibody Against the Active Form of Caspase 3 and Correlation with Tumour Histopathological Features

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Introduction: Caspase 3 is a cytosolic enzyme which is activated in cells undergoing apoptosis. Its activation precedes the classical morphological features of apoptosis. The aim of this study is to establish whether measurement of apoptosis by immunohistochemistry using an antibody against the active form of caspase 3 correlates with morphological assessment in 60 invasive breast carcinomas embedded in paraffin.

Methods: Consecutive sections were obtained and the apoptotic index(AI) was determined by both immunohistochemistry and morphological assessment. Histopathological characteristics of these tumours including Ki67 were examined following surgery.

Results: A strong correlation in the apoptotic indices was found between the two methods (Spearman's rank 0.842,$p<0.01$). The table below shows the medians and interquartile ranges of AI in the node positive and negative groups.

	n	AI(morphological)	AI(anti-active caspase 3)
Node positive	32	0.51(0.15-1.54)	0.51(0.15-0.82)
Node negative	28	0.11(0-0.65)	0.10(0-0.7)
P value		0.031	0.032

High Ki67 and negative steroid receptor status correlate with high apoptosis ($p=0.002$ and $p<0.02$ respectively).

Conclusion: Measuring apoptosis by immunohistochemistry using an antibody against the active form of caspase 3 is an accurate and reliable method. Higher AI is associated with more aggressive cancer features.

378 Exisulind Inhibits Cell Growth, Induces Apoptosis, and Has Synergy with Herceptin and Taxotere in Breast Cancer Cells.

Pegram MD,[1] Liu L,[2] Lloyd M,[2] Pamukcu R,[2] Slamon DJ,[1] Thompson WJ.[2] [1]Hematology-Oncology, UCLA School of Medicine, Los Angels, CA; [2]Cell Pathways, Inc., Horsham, PA.

Exisulind (Aptosyn™) is an oral pro-apoptotic drug that causes regression, and prevents formation of, new colon adenomas in patients with familial adenomatous polyposis (FAP) and prevents progression of prostate cancer as measured by PSA in post-prostatectomy with rising PSA. Exisulind inhibits cGMP phosphodiesterases and activates cGMP-dependent protein kinase to induce apoptosis in colon polyps and cultured tumor cells. The apoptotic effect is independent of COXI or COX II inhibition, p53, Bcl-2 or cell cycle arrest. In this study, we investigated the anti-proliferative and pro-apoptotic effects of exisulind alone and in combination with Herceptin or Taxotere on human breast tumor cell lines (MCF-7, MDA-MB-231, MDA-MB-453, MDA-MB-435S, MDA-MB-436, BT-20, BT474, SR-BR-3) with differential expressions of HER2/neu and estrogen receptors. Growth inhibition was determined after a 6 day exposure to the drug using sulforhodamine dye binding, and apoptosis after 2 day exposure using DNA fragmentation by double antibody ELISA. Exisulind showed an IC_{50} =32~248 μM for growth inhibition in all eight cell lines and was not dependent on HER-2/neu and estrogen-receptor expression. Exisulind showed a similar EC_{50} for apoptosis in MDA-MB-451 cells. Herceptin, though growth inhibitory with an IC_{50}=44 ng/mL and 50% maximal effect, did not induce apoptosis at 48 hour treatment. When studied with multiple drug effect/ combination index (CI) isobologram analysis, exisulind/Herceptin showed CI<1 in receptor over-expressing breast cells, indicating a synergistic effect for growth inhibition and apoptosis. Exisulind was also synergistic with Taxotere against breast cancer cells independent of HER2 status. These data provide support for clinical studies of exisulind as a single agent and in combination with Herceptin and/or taxanes in the treatment of breast cancers.

379 Induction of Apoptosis in Breast Cancer Cells by Inhibition of Glucose Metabolism.

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Accelerated glucose uptake for anaerobic glycolysis is one of the major metabolic changes found in malignant cells. This property of cancer cells has been exploited for imaging (PET) and as a possible anticancer strategy. We and others have found that Glut1 is the major glucose transporter expressed in breast adenocarcinomas and in breast cancer cell lines (MCF-7, BT474, MDA/MB468, SKBR3). To exploit glucose deprivation as anti-cancer therapy, we have inhibited glucose metabolism with the anti-metabolite 2-deoxyglucose (2DG). The putative action of 2DG is inhibition of the first enzymatic step in glucose metabolism, phosphorylation by hexokinase. Treatment of breast cancer cell lines with 2-deoxyglucose results in cessation of cell growth in a dose dependent manner. Treated cells are induced to undergo apoptosis as measured by caspase 3 activation, DNA degradation, and PARP activation. In addition, inhibition of glucose metabolism results in increased levels of Glut1 protein expression as measured by Western blot analysis. We hypothesize that breast cancer cells treated with 2DG accelerate their own cell death by initially expressing increased levels of glucose transporter protein which allows increased uptake of 2DG. We believe that inhibition of glucose metabolism can be developed into an effective treatment for breast cancer.

380 mRNA Expression, Localization and Genomic DNA Amplification of Decoy Receptor 3 (DcR3) in Breast Cancer.

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Fas/Fas ligand (FasL) system plays an important role in tumor cell death by cytotoxic lymphocytes (Cell 1997; 76: 355-365). Decoy receptor 3 (DcR3), sharing the extracellular motif of the TNFR family, shows inhibitory effect to Fas-mediated apoptosis (Nature 1998; 396 (6712): 699-703).

Role of DcR3 in tumorigenesis and pathophysiology of breast cancer has not been reported yet. We examined mRNA expression and genomic amplification of DcR3 to clarify its role(s) in human breast cancer.

Human breast tissues were obtained from 14 breast cancer patients. Total cellular RNA was isolated from these tumors. Partial cDNA fragments of DcR3, Fas and FasL were cloned by RT-PCR and used as templates for in vitro transcription of isotope-labeled antisense cRNA probes. mRNA expression of DcR3, Fas and FasL and mRNA localization of DcR3 were examined by ribonuclease protection assay (RPA) and *in situ* hybridization (ISH), respectively. Genomic DNA of cancer tissue corresponding patient was extracted from paraffin embedded section by microdissection under light microscope. Quantitative PCR was carried out by using a TaqMan (Applied Biosystems 7700) to determine genomic amplification of DcR3 being in breast cancer. Genomic amplification was defined as relative DcR3 copy number greater than 2-fold after normalization to the β-globin in the test DNA.

DcR3 mRNA expression were extensive in 5 cases (36%) and faint in 9 cases (64%) by RPA. Although Fas mRNA expressed was observed in the specimen that expressed DcR3, FasL mRNA could not be detected in all specimens. ISH revealed prominent expression of DcR3 mRNA in breast cancer cells. Although DcR3 gene amplification were observed in the same cases (5/14) which showed extensive DcR3 mRNA expression, not in the faint mRNA expression cases. These results suggest that breast cancer, in some part, express DcR3 under the gene amplification to evade the apoptotic mechanism.

401 Prognostic Impact of Mucin-Positive Cells in the Bone Marrow of 1338 Patients with Primary Breast Cancer.

Diel IJ, Solomayer EF, Hahn M, Gollan CH, Schütz F, Bastert G. Dept. of Ob/Gyn, University Hospital, Heidelberg, Germany.

The detection of disseminated tumor cells in the bone marrow of patients with breast cancer is associated with a poorer prognosis. These cells are also the focus of new treatment modalities. Because detection methods are not only diverse but have also usually been tested in only small groups, our aim was to investigate a detection method in a large number of patients under standardized conditions and with a long follow-up time.

Intraoperative, bilateral iliac crest biopsies were performed in 1338 patients with primary breast cancer. The aspirated bone marrow was subjected to differential centrifugation, smeared onto slides and stained with a monoclonal antibody that recognizes the MUC-1 gene and targets the tumor-associated glycoprotein TAG 12. Patients underwent follow-up examinations at regular intervals. The results were statistically evaluated.

After a median follow-up period of 56 months, distant metastasis was observed in 368 patients (28%). 225 (61%) were tumor-cell positive at the time of surgery. Of the 238 patients who died 161 (68%) were positive. In a multivariate Cox regressions analysis tumor cell detection was by far the best prognostic factor in patients with small breast tumors (T1). In women with tumors larger than 2 cm nodal status and tumor cell detection had the same prognostic value.

Our investigations show that the dissemination marker micrometastasis has a greater important for the prognosis of the disease in women with small breast tumors than the classic prognostic factors. In this group of patients it might be better to dispense with axillary lymphadenectomy in favor of tumor cell detection. The goal must be to destroy these individual cells in the bone marrow, which are not accessible to standard cytotoxic treatments, by means of a new therapeutic modality (immunotargeting, gene therapy, bisphosphonates).

402 Real Time Monitoring of the Efficacy of Adjuvant Therapy in Breast Cancer Quantifying the Reduction of Circulating Tumor Cells by MAINTRAC (Laser Scanning Cytometry of Magnetic Bead Enriched Cells).

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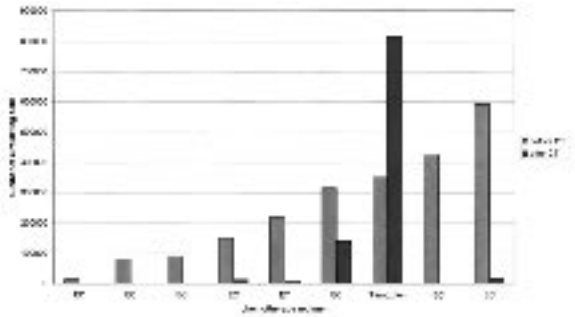
Metastases are the most common causes of cancer related death in breast cancer in women even in patients with early stage disease leading to a variety of strategies in adjuvant chemotherapy. It is assumed that metastases are due to tumor cells disseminated into the circulation. The aim of the present study was the quanti- fication of circulating suspicious cells and monitoring their reduction in response to the applied therapy. So far 100 breast cancer patients and 50 normal donors have been screened for circulating cells carrying epithelial antigens by the MAINTRAC approach. Red blood cells were lysed from 20ml of peripheral blood, the cells carrying epithelial antigen enriched using magnetic beads and stained for epithelial antigens using fluorochrome labeled antibody. This cell population was then measured in a laser scanning cytometer and positive events controlled visually. This allows to detect down to 10 tumor cells in 20ml of whole blood with a recovery rate of 90%. In 49 normal donors the only cells staining positively were skin epidermal cells. One healthy donor had varying numbers of positive cells and will be further observed. In breast cancer patients the numbers of epithelial antigen positive cells varied over 5 decades (from 103 to 107) per 5l blood volume. In 4 patients suspicious cells were undetectable. For 9 patients rescreening after the adjuvant chemotherapy regimen showed a reduction of circulating tumor cells all but one patient, treated with tamoxifen only, varying due to the initial stage and regimen applied (Fig 1). Thus the MAINTRAC approach allows for the first time directly a current individual control of the therapeutic effect in the adjuvant situation in solid tumors.



403 The Fate of Occult Metastatic Cells in Follow-Up Bone Marrow Aspirations of Patients with Primary Breast Cancer.

Janni WJ, Rjosk D, Hepp F, Kentenich C, Braun S. I. Frauenklinik, LMU Munich, Munich, Germany.

Our recent data demonstrated that occult metastatic cells in the bone marrow (BM) of patients with breast cancer (N Engl.J Med 2000;342:525-33) exert an important independent influence on patients' prognosis. Although we found an association of tumor cell dissemination with subsequent occurrence of bone metastasis, it is unknown whether micrometasstatic tumor cells merely reflect an epiphenomenon or actually persist in BM of those patients who relpase. To address this question, we have thus far analyzed BM aspirates from 89 breast cancer patients at the time of primary diagnosis, and after an interval of more than 6 (median, 20) months without further manifestation of disease. To identify epithelial tumor cells, we applied an immunoassay with the monoclonal anti-cytokeratin(CK) antibody A45-B/B3, and evaluated 2 x106 bone marrow cells per patient. In 24 of 89 patients (27%), occult metastatic cells in BM were detected at time of primary diagnosis, compared to 25 of 89 patients (28%) at the time of follow-up examination. Of those 65 patients with an initially negative BM finding, 15 patients (23%) presented with BM tumor cells at 6 or more months after surgery. Such tumor cells were also detected in 10 of 24 patients (42%) with an initially positive BM finding. After the so far short duration of clinical follow-up (median, 20 months), there appears to be a tendency towards a reduced interval of cancer specific survival (P= .13). Although a longer observation time will be needed for a meaningful clinical interpretation of our data, it is noteworthy that 28% of patients present with minimal residual breast cancer approximately 2 years after surgery. This observation strongly supports the hypothesis of prolonged dormancy of disseminated tumor cells, and is in accord with the well known clinical finding of the long latency between diagnosis and initial recurrence in breast cancer.

404 MHC Class I Expression of Metastatic Breast Cancer Cells Isolated from Peripheral Blood.

Pham DT,[1] Mosca PJ,[1] Clay TM,[1] Morse MA,[2] Vredenburgh JJ,[2] Ross AA,[3] Lyerly HK.[1] [1]Surgery, Pathology, Immunology; [2]Medicine, Division of Hematology-Oncology, Duke University Medical Center, Durham, NC; [3]Nexell Therapeutics Inc., Irvine, CA.

Background: A promising approach for eradicating minimal residual tumor burden may be the use of therapeutic vaccines to stimulate cytotoxic T lymphocytes capable of recognizing tumor cells. Tumor antigen recognition is mediated by T cell receptors specific for tumor antigens presented within the groove of the class I and II major histocompatability complex (MHC) molecules. One proposed mechanism by which cancer cells evade the immune system is by down regulating class I MHC molecules. Because we hypothesized that tumor cells circulating in the peripheral blood are the likely source for metastatic disease and that the success of an immune-based strategy in destroying micrometastases would depend in part on whether these cells expressed class I molecules, we sought to determine whether circulating metastatic breast cancer cells express high levels of class I MHC molecules. **Methods**: We obtained discarded peripheral blood mononuclear cell (PBMC) samples from seven patients with metastatic breast cancer undergoing cytokine mobilized peripheral blood stem cell collection by leukapheresis. Using the prototype Tumor Enrichment Column (TEC™; Nexell Therapeutics, Irvine, CA), we enriched circulating tumor cells from the patient samples. We confirmed that the enriched cells were tumor cells by detecting overexpression of HER2/Neu. We then determined the level of class I MHC expression on the HER2/Neu+ cells using anti-class I - A, B, C MHC mAb and flow cytometric analysis. **Results**: We enriched the circulating tumor cells as high as 205-fold to a purity as high as 14.33% of total nucleated cells. We also found that these circulating tumor cells expressed high levels of class I MHC (median 91.45% cells, median mean fluorescence intensity 63.94). **Conclusion**: These data suggest that cancer vaccines directed toward peptide antigens expressed by metastatic tumor cells may recognize these cells and may prove effective in reducing clinical metastasis.

405 Detection of Breast Cancer Cells in Bone Marrow and Peripheral Blood with Magnetic Bead Enrichment and Laser Scanning Cytometry (MAINTRAC).

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Detection of breast cancer (BC) cells in bone marrow (BM) has been reported as an independent prognostic indicator in breast cancer and is superior to lymphnode status, tumor stage and tumor grade for predicting overall survival and disease free survival. Other reports that BC tumor cells can shed into the circulation of BC patients. Still there is only few information about the significance of the present of BC cells in the peripheral blood (PB). This study compares the conventional immuncytochemical BC cells detection in the BM with the new method of quantitativ determination of the number of tumorcells using magnetic bead enrichment and laser scanning cytometry (MAINTRAC) and also using the same method for circulating BC cells detection in the blood.

BM samples obtained at surgery of 19 breast cancer patients were analysed by immunhistochemistry cytokeratin 88, 18, 19 and compared with the MAINTRAC method. From the same patients tumor cell numbers in PB were determined additionally. One half of the sample was analysed by immunochemistry and the other by MAINTRAC. Anti-human epithelialantibody (HEA)-magnetic beads for enrichment of tumor calls staining was performed with an FITC conjugate HEA antibody.

Correlation between MAINTRAC and conventional immunohistochemistry results for eleven patients were obtained. In 19 of 19 cases the immunhistochemistry method for breast cancer cells in the BM was negativ. In contrasts with the MAINTRAC method tumor cells can allways be proven in all cases of biopsy. The number of cells isolated is between 0,28 and 834/mill WBC (median 12/mill). The detection of BC cells in the PB was negative in only one patient. In the positive samples we found values between 1 and 1300 cells. In one case we found a clear BC cell reduction after neoadjuvant chemotherapy in the PB and in the BM. A correlation between tumorstage and lymphnodestage with the results of the BM and PB analysis was done.

MAINTRAC laser scanning cytometry provides a significant enhancement of the sensitivity for detecting cancer cells in the bone marrow and peripheral blood compared to the conventional immunocytochemistry method. It has yet to be determined in further studies whether the MAINTRAC method can better predict the prognosis of the disease.

406 An Ultra-Sensitive Tumor Enriched Immunocytochemical (EICC) Assay for Detection of Micrometastases in Blood of Breast Cancer (BrCa) Patients.

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As new therapies become available for breast cancer patients, it will be important to have tests that can monitor efficacy. Detection of bone marrow (BM) micrometastases has clearly shown prognostic value in identifying patients at high risk for metastatic disease. However, BM aspiration is an invasive procedure and physicians are hesitant to use these types of samples for serial testing.

We have developed an EICC assay that can detect routinely 1/10,000,000 tumor cells. With this assay it is possible to determine the presence of circulating BrCa cells without performing BM aspirates. The system utilizes Miltenyi's super-paramagnetic microbeads conjugated with an anti-epithelial (HEA-125) monoclonal antibody to enrich tumor cells prior to immunocytochemical analysis. In seeding experiments, CAMA cells were seeded into aliquots of PBSC from normal donors at concentrations of 1/1,000,000, 1/5,000,000, 1/10,000,000, 1/20,000,000, and 1/ 50,000,000. Overall tumor cell recovery in the positive fractions of the various seeded samples ranged between 56-97%. No tumor cells were detected in any normal controls. This EICC system was then used to enrich tumor cells from peripheral blood (PB) of BrCa patients. For 43 enriched PB samples of stages I-III and 38 of stage IV patients, positive cells were found in 12 (28%) and 15 (39.5%) patients, respectively. The incidence of BrCa cells found in the blood, using the EICC assay is similar to that found when analyzing BM. In addition, EICC testing also detected circulating BrCa cells in 17 of 31 stem cell products (54.8%) taken from chemosensitive patients. Tumor cell concentration ranged from 1/1,500,000 to 1/25,000,000.

In conclusion, the EICC assay now permits testing of PB for minimal residual cancer in BrCa patients. This ultra-sensitive assay may permit PB samples to be used in lieu of marrow and allow testing of serial samples for monitoring therapy.

407 Biochemical Markers of Bone Metabolism Predict Prognosis in Patients with Bone Metastases of Breast Cancer.

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We have shown that the bone metabolic markers, especially serum carboxy-terminal telopeptide of type 1 collagen (ICTP) and serum bone-specific alkaline phosphatase (BAP), were useful for diagnosis and follow-up of bone metastases of breast cancer (Int. J. Clin. Oncol. 4:331, 1999, Clinical Nuclear Medicine. 14:15, 1999). In this study, we evaluated the efficacy of the bone metabolic markers as prognostic predictor for patients with bone metastases. 415 patients with breast cancer who were examined by bone scintigraphy were enrolled for this study and 232 patients were diagnosed to have bone metastases. Urinary free deoxypyridinoline (fDPD, ELISA, Metrabiosystems), serum ICTP (RIA, Orion Diagnostica), osteocalcin (OC, RIA, Mitsubishi-BCL), procollagen type I C-terminal peptide (PICP, RIA, Orion Diagnostica) and BAP (IMA, Metrabiosystems) were measured. Values of all markers were significantly increased in patients with bone metastases than those in patients without bone metastases. The survival and the time to first skeletal-related events were calculated in patients with bone metastases. With median followup of 1183 days, time to first skeletal-related events (hypercalcemia, pathological fracture, operation and radiation) was significantly short in patients with elevated ICTP, BAP, and PICP. Overall survival of patients with elevated ICTP, fDPD, BAP, and PICP was also significantly worse (with ICTP, 473 days vs. 1225 days, $p< 0.0001$). Multivariate analysis for survival or skeletal-related events showed that ICTP, PICP and BAP were significant prognostic factors. Bone metabolic markers might be useful prognostic factors in patients with bone metastases of breast cancer.

408 Independent Prognostic Role of Changes in CA 15-3 Serum Level and Clinical Response after First Line Chemotherapy in Metastatic Breast Cancer (BC) Patients (PTS).

Berruti A, Tampellini M, Gorzegno G, Danese S, Durando A, De Matteis A, Genta F, De Fabiani E, Nuzzo F, Manzin E, Sarobba MG, Castiglione F, Moro G, Giardina G, Farris A, Massobrio M, Dogliotti L, and the EPI-LON Group. Orbassano, Torino, Italy.

To date, available data are insufficient to demonstrate a real utility of CA 15-3 in the management of BC pts. In order to explore a possible prognostic role of marker changes during first-line chemotherapy, we recorded data from 526 newly diagnosed metastatic BC pts included in 5 consecutive phase II-III trials conducted between October 1988 and November 1999. At baseline, 327 pts (62.2%) had 1 organ involved, 159 (30.2%) 2 organs involved and 40 (7.6%) more than 2; 169 recurred in soft tissue (32.1%), 261 (49.6%) in bone, 195 (37.1%) in lung and 140 (26.6%) in liver; supranormal CA 15-3 levels at diagnosis were found in 329 pts (62.5%). Clinical assessment and marker measurement were performed every 3 months. A decrease >25% was considered a marker response; stable marker was between response and increase >25%; marker progression was a >25% increase. 63.8% pts obtained a clinical CR+PR after treatment, 27.5% a NC, and 8.7% progressed. In 161 pts (30.6%) CA 15-3 levels were underneath the threshold at baseline and remained negative after treatment (CANE group), reduction >25% was recorded in 204 (38.8%) pts (CA25 group) and increased in 83 (15.8%) (CAP group). At the time of data computation, 259/526 pts (49.2%) died, with a median follow-up of surviving pts of 23.3 mo. Median survival for pts categorized according to CA 15-3 changes were: 43.6 mo for CANE, 29.7 mo for CA25, and 20.2 mo for CAP ($p<0.002$). Survival computations of pts with a clinical benefit from chemotherapy (CR+PR+NC) stratified according to CA 15-3 variations demonstrated a longer survival for the CANE group (42.1 mo) than the CA25 (29.7 mo) or the CAP (18.6 mo) ones ($p<0.001$). Multivariate Cox's analysis showed that clinical response and CA25 were independent prognostic indicators ($p<0.03$), whereas age, stage of primary tumor, bone and soft tissue as dominant metastatic sites failed to enter the model. To conclude, these data support the use of CA 15-3 in the clinical routine of advanced BC pts. Pts with baseline negative marker values persisting after treatment had a good prognosis independently from tumor response and marker reduction >25% was a predictor of better outcome.

409 Serial Monitoring of Serum HER-2/neu in Women with Metastatic Breast Cancer Correlates with the Clinical Course of Disease.

Carney WP, Hamer PJ, Tenney DY, Johnson KA, Allard WJ, Yeung K, Neumann R, Brown-Shimer S. Oncogene Science/Bayer Diagnostics, Cambridge, MA.

Our studies have shown that the HER-2/neu extracellular domain (ECD) is shed into the serum of normal women and is elevated in women with metastatic breast cancer (MBC). These studies have been conducted with a standardized ELISA, which was specifically validated for measuring the ECD in human serum. In a recent study evaluating the HER-2/neu oncoprotein as a tool to monitor women with MBC, we have used the serum ELISA to analyze serial samples from over 100 women with MBC. In addition, samples from over 200 normal women (pre- and post-menopausal) and women with benign breast diseases or non-malignant, non-breast diseases were tested. The upper limit of normal was established at 15 ng/mL and the longitudinal variability was less than 20%. Longitudinal changes of serum HER-2/neu ECD were found to correlate with changes in disease status in greater than 80% of the cases. Serial increases of 20% or greater from previous determinations were indicative of progressive disease, while serial decreases of 20% or greater from previous determinations were reflective of a response to therapy or a lack of progression during therapy. Our studies also demonstrated that some women with MBC who had elevated ECD serum levels did not respond as well to hormone therapy as women with normal serum HER-2/neu levels. We also evaluated HER-2/neu serum levels in patients with MBC both before and after Herceptin therapy. Prior to Herceptin therapy three patients demonstrated very high serum levels of the HER-2/neu ECD (greater than 100 ng/mL), which decreased dramatically to 15 ng/mL or less with clinical response to Herceptin. Herceptin spiked into serum samples did not interfere with recovery of the HER-2/neu ECD in the ELISA. Our studies also showed that the microtiter ELISA correlated extremely well with the automated Immuno-1 version of the HER-2/neu assay. Quantitation of the serum HER-2/neu ECD may have several clinical applications, such as monitoring women with metastatic breast cancer or predicting response to hormone therapy. In addition, serum HER-2/neu measurements provide a real-time opportunity to assess a woman's HER-2/neu status rather than depending on a semi-quantitative retrospective tissue analysis.

410 The Use of Blood Tumour Markers in the Monitoring of Metastatic Breast Cancer Unassessable for Response to Systemic Therapy.

Cheung KL, Evans AJ, Chan SY, Robertson JF. Nottingham Breast Unit, City Hospital, Nottingham, United Kingdom.

The role of blood tumour markers is established in the monitoring of response to systemic therapy for patients with metastatic breast cancer assessable by UICC criteria. This study examines the use of marker measurements (in the form of a previously devised biochemical index score comprising CA15.3, CEA and ESR) in patients with metastatic lesions unassessable for response by UICC criteria.

Of 218 patients with metastatic breast cancer treated in the Nottingham Breast Unit over a two-year period, 43 patients (20%) had unassessable disease: 29 of them with both pre-treatment and sequential blood marker results available were studied. Twenty-four patients (83%) were biochemically assessable during the course of treatment. All patients who had biochemical response (n = 10) remained unassessable by UICC criteria. Eighteen patients progressed either biochemically or by UICC criteria. Biochemical assessment completely paralleled UICC assessment in all 12 patients who progressed by both assessments: in six of them biochemical progression occurred ahead of UICC assessment with a median lead-time of 3.5 months.

Biochemical assessment by blood tumour markers is useful in patients with metastatic breast cancer unassessable for response to systemic therapy.

411 Identification of Serum Nuclear Matrix Protein Markers of Breast Cancer Using Surface Enhanced Laser Desorption-Ionization (SELDI) Mass Spectroscopy.

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Analyzing serum using a combination of liquid chromatography (LC) and SELDI-MS we were able to fractionate each IgG/HSA depleted serum sample into 60 different MS traces defined by the LC fraction and SELDI chip surface used to obtain the trace. Applying this process to a series of twenty pre-operative samples from patients with pathologically confirmed breast cancer, and twenty samples from women over 40 with no history of breast disease, we were able to identify a series of 15 peaks that were present in at least 75% of the cancer samples but absent in at least 75% of the normal samples. Of these 15 peaks, 3 were present in all 20 cancer samples and in none of the normal samples. These 3 proteins were identified by purifying each from a large volume of serum, and analyzing the sequence of tryptic fragments using tandem MS. This analysis identified 2 of the 3 markers as nuclear proteins. Analysis of 30 additional serum samples has shown that these three markers are present at all stages of breast cancer and preliminary data indicates that they are generally not present in cases of benign breast disease.

412 Correlation of Shed Serum HER-2/neu and Tumor Marker CA 15-3 with Response to Chemotherapy for Metastatic Breast Cancer.

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Introduction: Shed extracellular domain (ECD) of the HER-2/neu oncogene product can be detected in serum. This study evaluated the potential use of this protein to monitor therapy in patients with breast cancer. In a retrospective analysis the correlation between HER-2/neu serum concentration and tumor marker CA 15-3 with therapy response in patients treated for metastatic breast cancer was examined.

Methods: Serum of patients with metastatic breast cancer (n=46) participating in a clinical trial on first-line chemotherapy was collected before initiation and after 3 courses of therapy. Epirubicin 60 mg/m2 and cyclosphophamide 600 mg/m2 (EC) or epirubicin 60 mg/m2 and paclitaxel 175 mg/m2 (ET), were given in 3-week intervals. Healthy blood donors were taken as control to evaluate normal values (n=30). Concentration of HER-2/neu (p105) extracellular domain (ECD) in serum was determined using a commercially available ELISA (Oncogene Science Diagnostics). Tumor marker CA 15-3 was determined using a standard ELISA (IMx CA 15-3 by Abbott).

Results: All controls had ECD serum levels below 12ng/mL. Patients with metastatic disease had ECD-levels between 6 and 800ng/mL (median 12.73ng/mL). ECD-levels in the study group were elevated in 51.5% before therapy. The median serum level dropped to 11.4ng/mL in patients with complete or partial remission (CR/PR). In 70% of patients with CR or PR after 3 courses of chemotherapy, the serum level decreased. A decrease of ECD levels was associated with response to therapy (p=0.006, Wilcoxon-Test). Tumor marker CA 15-3 levels were elevated (>30U/mL) in 69.4% of patients before initiation of therapy. Median levels of CA 15-3 were 58U/mL before and 38U/mL after therapy, also correlating with therapy response (p=0.002, Wilcoxon-Test) and decreasing in 86% of patients with CR or PR.

Conclusion: A decrease of HER-2/neu oncogene product ECD and tumor marker CA 15-3 in serum of patients with metastatic breast cancer correlates with response to chemotherapy.

413 Serum Levels of Circulating Intercellular Adhesion Molecule-1 (s-ICAM-1) in Patients with Breast Cancer before and after Surgery: Correlation with Pathological Parameters.

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Increased s-ICAM-1 levels have been found in the circulation of patients (pts) with different malignancies and are implicated in tumor progression and metastasis. Few reports, however, deal with the relationship between the levels of s-ICAM-1 and breast cancer, with controversial results, and none with levels before and after surgical removal of the lesion.

This study evaluates and compares the pre and post operative (6 days after) levels of s-ICAM-1 of 16 pts with breast cancer using an immunoenzyme assay approach. Eight pts with fibrocystic disease were used as controls and their s-ICAM-1 levels were evaluated pre-operatively. **Before surgery**: levels of s-ICAM-1 were detected in 14 out of 16 cancer pts and in all pts with fibrocystic disease. No correlation of s-ICAM-1 levels with age, pathological parameters and receptor status was observed within the cancer group. No differences in s-ICAM-1 levels were found between pts with cancer and fibrocystic disease (mean s-ICAM-1 concentrations were 322.0 ng/ml and 358.5 ng/ml respectively). **After surgery**: levels of s-ICAM-1 were detectable in 14 pts. Their comparison before and after surgery showed: i) a decrease in all 5 pts with vessel invasion and in 1 patient with pre-operative chemotherapy and no vessel invasion. ii) an increase in all 5 pts with positive auxiliary lymph nodes. iii) Four pts with negative lymph nodes showed either increase (2) or decrease (2). iv) One patient with pre-operative chemotherapy had undetectable s-ICAM-1 levels both pre and post operation.

Our results, although restricted to 24 pts, suggest that s-ICAM-1 levels could not identify pts with benign or malignant breast lesions. There are no statistically significant differences in s-ICAM-1 levels before and after surgical treatment. The observed post surgical decrease in s-ICAM-1 levels in cases with vessel invasion is interesting. Further investigation is needed to elucidate a possible relationship of s-ICAM-1 and breast cancer with vessel invasion.

414 Histologic Grade and ER/PgR Status in Breast Carcinoma.

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Background: Estrogen (ER) and progesterone (PgR) receptors expression in breast cancer provides both predictive and prognostic information. This correlation has been reported in the literature primarily from studies using ligand-binding assays. Although ligand-binding assays (LBA) has been the gold standard, immunohistochemistry (IHC) is now the commonly used method of detecting ER/PgR. Histologic grade of breast cancer, also a predictor of disease outcome, has been shown to correlate with ER status. However, this data was based on LBA. IHC has now replaced LBA as the method of choice for determining ER status. In this study, we therefore examined the relationship between ER status and histologic grade in breast tissue optimally fixed alcoholic formalin.

Design: Histologic grade (Scarff-Bloom-Richardson system) and ER/PgR status in 473 alcoholic formalin (Ethanol:37% formalin = 1:1) fixed cases of breast carcinoma was examined. Hormone receptor positivity was assessed by two methods; percent of positive cells and HS score (Histoscore). ER positive status was defined as either of ER or PgR positivity.

Results: The distribution of histologic grade in 473 cases was grade I 20%, grade II 33.0%, and grade III 47%. Correlation of ER status & histologic grade is shown below.

	HS score (>74)		Percent (>20%)		ER status	
Grade	ER	PgR	ER	PgR	HS score	Percent
I	92.6	52.6	96.8	65.3	93.7	98.9
II	92.9	55.8	94.2	60.9	92.3	94.2
III	49.5	26.1	49.5	27.5	51.0	51.8

Conclusions: There was no significant difference in ER status determined by percentages or HS score methods. Greater than 90% of tumors with histologic grade I or II were ER status positive, suggesting that it may be unnecessary to perform ER/PgR estimation in these tumors. The higher incidence of ER positive status in this study may be related to better fixation using alcoholic formalin. False negativity due to poor fixation could explain response to hormonal therapy in some ER-negative tumors.

415 HER2 Overexpression in Breast Cancer: Correlation between Quantitative Expression Data and Clinical Diagnostic Tests.

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To date, few studies compare FDA approved tests with quantitative HER2 expression data. We examined predictibility & reproducibility of IHC (CB11 & Herceptest) & fluorescence in situ hybridization (FISH) vs. quantitative HER2 expression (QIHC) in 216 breast carcinomas. Tests were scored by two observers.

By QIHC 20.4% of cases showed >10x normal HER2 expression levels. By CB11 Scorer A categorized 70.9% of cases 0/1+ (151) and 29.1% as 2/3+ (63 cases) vs. 53.0% 0/1+ (113) and 47.0% as 2/3+ (97 cases) scorer B. Disagreement would have altered treatment in 38 cases (Kappa = 0.74). Using the Herceptest scorer A categorized 176 cases as negative vs. 158 for scorer B. Only 28 cases were 2/3+ (scorer A) vs. 26 cases (scorer B). In only 19 cases did both select for treatment (8.5%, Kappa = 0.67). In 172 cases the HER2:Chromosome 17 ratio was <2.0 (non-amplified) whilst in 42 (19.44%) cases the HER2:Chromosome 17 ratio was >2.0 (amplified, Kappa = 0.973).

Whilst the DAKO antibody showed a higher positive predictive value (PPV = 92.9%) a low negative predictive value (NPV = 89.8%) compromised the overall accuracy of this test (87.4%). Conversely the CB11 antibody showed a low PPV (63.2%), and a high NPV (94.7%, accuracy = 83.8%). Overall, when assessed against Q-IHC data, FISH testing provided the most accurate (NPV = 95.3%, PPV = 88.1%, accuracy = 93.2%) means of detecting quantitatively determined HER2 overexpression in this series of breast cancers.

In conclusion: The most accurate system for detection of overexpression of HER2, given the restraint of paraffin embedded tissues, appears to be quantitation of gene amplification (here by the Pathvysion system). In addition this system shows markedly lower observer error than IHC. Whilst strongly positive staining with the Herceptest is strongly predictive of overexpression and gene amplification, there is in this study, as in the recently reported comparison between the Herceptin clinical trials assay and FISH, a small proportion of gene amplified cases which are not scored positive by the Herceptest. It would therefore appear that FISH testing prior to patient treatment stratification based on HER2 status is advisable.

416 Comparison of HER-2/neu Analysis Using FISH and IHC When HercepTest® Is Scored Using Conventional Microscopy and Image Analysis.

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The concordance between fluorescence in situ hybridization (FISH) and immunohistochemistry (IHC) for the evaluation of HER-2/neu was compared in 129 breast cancer cases obtained from multiple institutions on a random basis. All cases were IHC stained using DAKO HercepTest® and also stained and scored for FISH. Eight pathologists were chosen to represent a range of experience from the most experienced (>50 Herceptest cases read per day) to comparatively inexperienced at reading HercepTest manually for a total of 971 manual reads. Concordance between FISH and manual IHC analysis strongly correlated with the experience of the reviewer and ranged from 38% to 82%. When 4 of the pathologists (including the most and least experienced) read the same slides using an image analyzer (ChromaVision ACIS), a total of 471 image analysis-assisted reads, the concordance increased to a range of 84% to 86%. Therefore, in these studies, the least experienced pathologist reading IHC with assistance of the ACIS had a higher concordance with FISH than the most experienced pathologist reading manually. In 13 specimens in which there was disagreement between ACIS-assisted IHC analysis and FISH, there was complete agreement between the 4 pathologists on the IHC score. These 13 specimens therefore appear to represent true biological- or methodological-based differences, and do not represent variability in interpretation. When these 13 specimens are excluded, the average concordance between FISH and assisted IHC is 96% compared to an average concordance of only 66% when the same specimens are read by manual microscopy. Based on the high concordance rate between FISH and IHC assessment by ACIS-assisted analysis, variations in sample preparation and staining play a much smaller role in IHC variations than was previously believed. Finally, we conclude that when computerized image analysis is used to assess IHC staining, the results are highly concordant with FISH analysis. This observation may eliminate the perceived need for staining and analyzing specimens by both IHC and FISH, an approach which is both laborious and expensive.

417 HER-2 Copy Number or HER-2:Chromosome 17 Ratio – Which Gives the More Accurate Measure of Overexpression?

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HER-2 status is a prognostic factor in breast cancer. Accurate assessment of copy number is crucial to treatment regimes. Known HER-2 protein levels in a cohort of primary invasive breast carcinomas, measured by quantitative IHC was compared to gene copy number with a HER-2 DNA probe versus HER-2:chromosome 17 ratio. Two hundred sixteen breast carcinomas were studied. Five micron archival tissue sections were used for the analysis with the Pathvysion test (Vysis, UK). Two independent observers scored 60 nuclei for both gene and chromosome copy number in each carcinoma.

Normal mean copy number per nucleus for HER-2 was 1.46 to 1.68 copies; for chromosome 17, 1.35 to 1.85 copies. Mean gene:chromosome ratio was 0.92 to 1.04. Fifty one (23.6%) breast carcinomas had >4 copies of HER-2 per nucleus and thus had abnormal gene copy number. In 39 of these 51 cases, aneusomy of chromosome 17 was also detected. Forty two (19.4%) breast carcinomas were amplified when measured by gene:chromosome ratio. In the nine non-amplified cases measured by ratio, aneusomy chromosome 17 was also detected.

Quantitative IHC assessment (Q-IHC) of HER-2 levels showed that of the 44 carcinomas with >10 fold normal HER-2 expression, associated with poor prognosis, 41 had gene copy number >4, a further 10 with <10 fold normal HER-2 expression also had >4 gene copies. Of the 42 amplified carcinomas, 38 exhibited >10 fold normal HER-2 expression. A further 2 carcinomas with >10 fold overexpression exhibited >10 copies of both HER-2 and chromosome 17.

Concordance of results between Q-IHC and FISH was higher for gene:chromosome ratio than for HER-2 copy number only (kappa = 0.93 vs. 0.91; overall accuracy = 94.2% vs. 92.6%). The gene:chromosome ratio had a higher consensus between observers than gene copy number alone (0.93 vs. 0.91). Gene:chromosome ratio was a stronger negative prognostic factor than HER-2 copy number alone (Hazard ratio 3.74 vs. 2.35 respectively): positive predictive value of ratio was 90.5% compared to 80.4% for HER-2 copy number.

In conclusion, assessment of HER-2:chromosome 17 ratio provides the more accurate prediction of protein over-expression. This may be due partly to the high frequency of chromosomal aneusomies in this patient cohort.

418 A Sequential Double Label Immunohistochemical (IHC) Technique for the Simultaneous Evaluation of Nuclear and Membrane Markers in DCIS and Invasive Breast Cancer.

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We describe an immunohistochemical method that allows evaluation of protein expression of two markers simultaneously on the same section of breast tissue. This sequential procedure, involving back-to-back IHC assays, can preserve precious tissue and allows evaluation of related markers in the same cells in the same tissue section. The basic method provides a framework that can be applied to numerous combinations of nuclear, membrane, cytoplasmic, stromal or extracellular matrix (ECM) markers and uses commercially available reagents and IHC kits. We have performed this technique on over 700 DCIS and invasive breast cancer specimens and have evaluated the following marker combinations: MIB-1(Ki-67) and Her2neu; estrogen receptor and E-cadherin; p53 and CD34. Important aspects that must be considered in the procedure include the following: 1). The two markers of interest must be localized to different regions of the cell or tissue (eg. Nucleus/membrane; nucleus/cytoplasm; cytoplasm/ECM); 2). Two different and contrasting chromogens must be used for detecting the two markers; 3). Antibodies directed against nuclear antigens or those requiring antigen retrieval are applied second in the sequential method. A key step in the technique, which prevents cross-binding of antibodies and reagents, involves acid treatment (.2N HCL) and post-fixation in zinc formalin performed between the two sequential assays.

The basic method involves the following: we first perform an IHC assay using the antibody directed against the membrane or cytoplasmic antigen; we next perform the IHC assay using the antibody directed against the nuclear antigen. The avidin-biotin immunoperoxidase method (ABC Elite kit, Vector labs®) is used frequently to detect both primary antibodies, although other detection systems provide good results. Many chromogen combinations were tested; the best contrast was obtained using Vector SG® (dark gray color) for membrane antigens and 3',3' diaminobenzidene (DAB; red-brown color) for nuclear antigens. Nuclear fast red and methyl green provide good counterstains.

Conclusion: This is a simple dual assay technique that can preserve precious tissue and allow evaluation of biologically relevant markers in the same breast cells using one tissue section.

419 Chromogenic In Situ Hybridization (CISH): A Practical New Alternative to FISH to Detect HER-2/neu Amplification in Archival Breast Cancer Samples.

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Determination of HER-2/neu oncogene amplification has become necessary for selection of breast cancer patients for trastuzumab (Herceptin) therapy. Fluorescence in situ hybridization (FISH) is currently regarded as a gold standard method for detecting HER-2/neu amplification, but it is rather expensive and not very practical for routine histopathologic laboratories.

We evaluated a new modification of in situ hybridization, the chromogenic in situ hybridization (CISH), which enables detection of HER-2/neu gene copies with conventional immunohistochemistry-like peroxidase reaction. Archival formalin-fixed paraffin-embedded tumor tissue sections were pretreated (by heating in microwave oven and using a short enzyme digestion) and hybridized with a digoxigenin-labeled HER-2/neu probe. The probe was detected with anti-digoxygenin-fluorescein, anti-fluorescein-peroxidase and diaminobenzidine. Gene copies visualized by CISH could be easily distinguished with 40x objective in hematoxylin-stained tissue sections. HER-2/neu amplification typically showed up as large peroxidase-positive intranuclear gene copy clusters. CISH and FISH (Vysis, made from frozen pulverized tumor samples) correlated well in a series of 157 breast cancers (kappa coefficient 0.81). The few different classifications were mostly due to low-level amplifications by FISH which were negative by CISH and p185 immunohistochemistry with monoclonal antibody CB-11.

We conclude that CISH, using conventional brightfield microscopy in evaluation, is a useful alternative for determination of HER-2/neu amplification in paraffin-embedded tumor samples, especially for confirming the immunohistochemical staining results.

420 Analysis of the Potential Contribution of the Estrogen Receptor (ER) β in the ER Cytosolic Protein Assay of Breast Cancer.

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Estrogen receptor (ER) content is the most useful parameter to predict hormone response therapy of breast cancer. Available assays for detecting ER in breast tumor cytosol include ligand binding assay (LBA), analyzing both ERα and ERβ or the enzymatic immuno assay (EIA), using monoclonal antibodies directed against the ERα. As shown in several studies, the two assays correlate well but some discrepancies exist and explanations remain controversial.

We evaluated ERα and ERβ mRNA coexpression in breast tumors in order to study whether the presence of ERβ could account for differences between LBA and EIA ER determinations. Using HeLa cell lines transfected by either ERα or ERβ we confirmed that the EIA assay, using H222 and D547 monoclonal antibodies, recognizes only ERα expression, whereas LBA detect both isoforms. In 119 breast tumors cytosols, the correlation between EIA and LBA ER determination was high (r=0.72) although some discrepancies were found. When analyzing REs mRNA expression of samples with LBA values higher than EIA ones, no overexpression of ERβ mRNA relatively to ERα mRNA were observed. There was a diffence in ERβ/ERα ratio between ER negative and ER positive samples, with a ten fold increased median ratio being found in ER negative samples (p=0.01).

We thus confirmed that the major form of ER in breast tumor is the ERα at both protein and mRNA levels. Moreover, our data does not support the hypothesis that ERβ expression could explain differences between LBA and EIA for ER determination.

421 Can Gene Expression Pattern Analysis Predict Recurrence in Node-Negative Breast Cancer?

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Some breast cancers spread (metastasize) to distant sites, putting the patient at high risk of death from this disorder. Clinicians now use tumor size, tumor appearance, and especially the presence of metastasis (cancer spread to local lymph nodes, or "node-positive breast cancer") to estimate the risk of early breast cancer death. These measures are imperfect, since 30% of the patients who should have a good outcome (no cancer spread to local lymph nodes, or "node-negative breast cancer"), eventually recur and die of breast cancer. Most low-risk node-negative breast cancer patients receive the same drug therapies routinely given to high-risk node-positive patients, i.e., most of the low-risk breast cancer patients are receiving treatments they do not need.

Our objective is to identify biomarkers that better define the metastatic potential of a node-negative breast cancer. Our tumor bank contains frozen breast cancers with information about whether the patient was cured by surgery alone, or, whether the tumor metastasized and led to the patient's death. Twenty breast cancer samples, all from untreated node-negative breast cancer patients are being tested on Clontech Atlas Human Cancer Array 1.2 cDNA arrays to compare the gene expression patterns. At present, we are blinded to the outcome of these tumors. When the data are complete and the code broken, we will use principle components methods to statistically evaluate differential patterns of gene expression between the never-metastasized and did-metastasize groups. Antibodies for these differentially expressed genes will be tested on archival specimens with known outcome to validate their prognostic significance. With this information, clinicians could identify either node-negative patients who require additional drug therapy for their disease, or could avoid over-treating those patients with very low risk of metastatic disease.

422 HER2/*neu* Alterations in Breast Cancer: A Comparative Study of Immunohistochemistry Using Three Antibodies and Quantitative PCR.

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Background: HER2/*neu* alterations are being routinely assessed in many labs, however, there is little consensus on which methodology or reagents are most appropriate to use in the clinical setting. The purpose of this study was to compare HER2/*neu* protein overexpression by immunohistochemistry (IHC) with HER2/*neu* gene amplification by quantitative PCR.

Materials and Methods: 254 invasive breast cancers were prospectively evaluated. DNA was extracted from a 10-micron tumour section for PCR and 4 micron serial sections were taken from the same block for IHC. The 3 antibodies evaluated were HercepTest (Dako), CB11 (Novocastra) and TAB 250, (Zymed labs). The results were scored using a semiquantitative IHC scoring system (J. Natl. Cancer Inst: 85, 1993). A positive tumour by IHC had a score ≥5. The manufacturer's recommended scoring system (0-3+) was used for the HercepTest. Tumours were positive for gene amplification if the ratio of the HER2/*neu* gene / control gene after normalization was ≥2.

Results: Sixty-one of 254 (24%) cases showed gene amplification. There was a statistically significant difference in HER2/*neu* amplification with respect to grade; 8% grade I/II, 16% grade II/III and 32% grade III/III tumours showed amplification, p=0.001. Using IHC, 23% of tumours were positive with CB11, 27% were positive with TAB250 and 37% were positive with the HercepTest. The results for each antibody were compared with the PCR results. The overall concordance for the HercepTest was 82%, which was significantly lower (p=0.01) than that for CB11 (88%) or TAB250 (89%). The specificity for the HercepTest was 80% compared to 90% for TAB250 and 93% for CB11, while the positive predictive value (PPV) for the HercepTest was 57% compared to 71% and 76% for TAB250 and CB11 respectively.

Conclusion: A higher percentage of cases showed HER2/*neu* immunopositivity with the HercepTest. The other IHC tests exhibited higher specificities and PPVs relative to the PCR results. Additional studies with clinical end points are required to determine which tests of HER2/*neu* status are most useful clinically in providing prognostic/ predictive information.

423 Time to Non-Breast Metastasis for Node Negative Breast Cancer Patients Who Received No Adjuvant Therapy.

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Many node negative breast cancer patients currently receive adjuvant therapy. Data for a cohort of 331 first primary node negative patients accrued 1971-90 and followed to early 1996, who received no adjuvant radiotherapy, hormonal therapy or chemotherapy, were used to model, with a log-normal survival analysis model, the time to non-breast metastasis. This cohort comprised 169 pathologically node negative (PN-) patients, 5 of whom were detected to have first recurrence regionally at median 18.5 months (range 12-51.5 months) after surgery, and 162 clinically node negative (CN-) patients, 15 of whom were detected to have first recurrence regionally at median 33 months (range 4.5-82 months) after surgery. Analyses were performed both with and without the 20 PN- and CN- patients who experienced regional recurrence due to the potential for confounding from original disease, although there were no significant differences in regional/distant DFS, or distant DFS, with/without these patients (p=0.44, 0.85). As well, there were no differences in DFS between the 181 patients who underwent lumpectomy versus the 150 who underwent simple/ subcutaneous/modified radical mastectomy (p=0.89, 0.81). The multivariate effects of age, tumour size, biochemical ER and PgR on DFS were examined; age (p=0.02) had a significant effect on regional/distant DFS, while age (p=0.03) and tumour size (p=0.10) had significant effects on distant DFS. There were 213 T1 tumours. The median time to distant recurrence (with 95% confidence limits) is the following: for a 40 year old woman with 1cm, 2cm, and 5cm tumours, it is respectively 33.2 years (0.8,>50 years), 26.3 years (0.6,>50 years), 13.1 years (0.3,>50 years); for a 50 year old, 45.1 years (1.1,>50 years), 35.7 years (0.8,>50 years), 17.8 years (0.4,>50 years); for a 65 year old, >50 years (1.7,>50 years), > 50 years (1.3,>50 years), 28.1 (0.7,>50 years). With predominantly small tumours, the median distant DFS is long, although the 95% confidence limits are very wide; better refinement would be obtained with more prognostic factors.

424 Angiogenesis Index (AI) Is Associated with Early Recurrence in Patients Presenting with Primary Breast Cancer.

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Angiogenesis index (AI) score within tumor specimens has been found to be significantly associated with progression to invasive phenotype and survival in invasive breast cancer (Proc. Am. Assoc. Cancer Res. 1997; 38:234, abst 1578). The AI score is the sum of index scores for p53 (greater expression being unfavorable), thrombospondin-1 (TSP-1, greater expression being favorable), and angiogenesis (greater blood vessel density by CD31 expression being unfavorable). AI values range from -12 to +3, with the higher values considered favorable. We determined the AI and collected biomarker data and follow-up information on 95 patients with T1-4, N0-2, M0 breast cancer prior to initiation of systemic therapy. Variables collected included age at diagnosis, tumor size, tumor grade, lymph node status, and estrogen and progesterone receptor status (ER, PR). An AI cut-point of ≤-6 previously validated on an independent cohort of breast cancer patients was applied to dichotomize the patients in this study into low and high AI groups. With a median follow-up of 91 weeks, 18/95 (19%) of patients have recurred; 6/69 above and 12/26 below the AI cutpoint, respectively (P<0.001; Fisher's exact test). Kaplan-Meier survival estimates show significantly shorter time to recurrence for patients with a low AI score compared to a high AI score (P=0.001; Log-rank test). Two year actuarial estimates for freedom from recurrence were 64% vs 91% in the low and high AI score groups. By univariate analysis, other factors correlating with recurrence included age, tumor size, lymph node status, and ER and PR expression. By multivariable analysis, factors retaining significance included AI, ER expression and tumor size. Low AI scores are associated with recurrence in primary breast cancer.

425 Differential Event Forecasting and Improved Risk Assessment in Breast Cancer Using Neural Networks.

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Recent approaches to individualized therapy in breast cancer require not only high performance in risk assessment and patient classification with respect to disease-free survival (DFS), but also differential forecasting of the relative probability of different types of metastasis, e.g., loco-regional recurrence, liver, or bone metastases. Studying the explanatory relationship between observed modes of metastasis and primary markers may offer more insight into disease processes typically associated with particular markers than only examining relapse and / or survival. If distinct disease processes are associated with particular combinations or interactions of factors, then intelligent nonlinear approaches such as CART or neural nets are the techniques of choice. This paper presents first results in such differential forecasting using a neural network environment incorporating likelihood-based optimization and complexity reduction techniques developed in our group. The combination of tumor biological factors (uPA, PAI-1) and neural network modeling led to improved risk assessment with regard to different endpoints such as DFS or relapse at individual locations in a group of 745 primary breast cancer patients. For example, using the median score derived from a neural network to classify patients as high or low risk for bone metastasis, only 3 % of the resulting low-risk group suffered bone metastasis within 5 years compared to 23 % in the high-risk group. After 10 years, the corresponding percentages were 7 % and 31 %. Improved differential event forecasting and patient risk assessment by neural networks could promote advances not only in developing individualized therapeutic concepts but also in designing therapy trials by choosing appropriate patient collectives.

426 Evaluation of an Automated System for Scoring Immunohistochemical Staining of HER2 in Breast Carcinoma Using Two Antibodies to HER2.

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Human epidermal growth factor receptor 2 (HER2) is overexpressed, in ~30% of breast cancers and is associated with a worse prognosis. Herceptin has been licensed for advanced breast cancers that overexpress HER2. The HercepTest has been approved by the FDA for determining who may benefit from this treatment. Patients whose tumours stain intensely (2+ by HercepTest) are considered suitable. An image analysis system may be useful for assessment of stain intensity.

We examined HER2 in formalin fixed tissue from a cohort of patients who were treated for breast cancer between 1984-1998 and for whom prospective follow up data was available. All of the tumours were stained for HER2 using the HercepTest and the CB11 monoclonal anti-HER2 antibody. The tumours were scored for HER2 expression by two pathologists using criteria set out in the HercepTest kit. All of these slides were re-scored with the Chromavision Automated cell imaging system(ACIS).

Of 24 cases that had a score of 2+ when stained with the HercepTest and scored manually, 19 gave a score of 2+ and 5 gave a score of <2 with the ACIS. All of these 5 scored >1 and were overexpressers of HER2 by quantitative immunohistochemistry (QIHC). Of 179 cases which scored <2 when stained with the HercepTest and scored manually, 174 scored <2 with the ACIS and 5 scored 2+. In 4/5 cases one of the pathologists gave the tumour a score of 2. Also 4 out of 5 were overexpressers of HER2 by QIHC.

Of 52 cases that had a score of 2+ when stained with the CB11 antibody and scored manually, 42 scored 2+ and 10 scored <2 with the ACIS. Only one of these was an overexpressor by QIHC. Of the 125 cases which had scores of <2 when scored manually, 92 scored 2+ and 33 scored <2 with the ACIS. None of these 33 were overexpressers when analysed by QIHC.

Ductal carcinoma in situ (DCIS) was often highly positive for HER2, by manually selecting scoring areas DCIS was excluded from analysis. Overall results from the HercepTest closely matched those from manual scores. Those from CB11 were higher possibly due to background staining. By choosing a higher cut off for treatment with this antibody stain this effect could be counteracted. A trained observer is necessary to choose the areas for scoring to ensure tumour is being scored and not DCIS.

427 Alterations of the Luteal Heat Cycle in Cancer-Associated Breasts.

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Breast surface temperature rises during the luteal phase of the menstrual cycle to a greater extent than core body temperature. We wished to test the hypothesis that this breast specific temperature might be higher in women at increased risk of breast cancer. We have therefore compared the breast temperature throughout the menstrual cycle in 12 control breasts and in 10 breasts at risk related to contralateral breast cancer.

Patients and methods: The Brassieres fitted with 12 temperature sensors were supplied by Professor Hugh Simpson in Glasgow. The sensors were worn for 90 minutes each evening through one luteal phase. Subjects also measured their daily oral temperature and collected daily saliva for progesterone assay. Breast temperature data were analysed by fitting a cosine function with a period of 14 days to the grouped data enabling the two series to be summarised into three parameters - phase, amplitude and level. Cycles were synchronised based on progesterone peak. Two sets of data from the controls were excluded due to assumed instrument fault or poor subject compliance.

Results: The contralateral breasts of patients with a history of breast cancer showed a higher mean breast surface temperature (35.90° C) than the controls (35.41° C), the amplitude of cyclical temperature fluctuation was significantly reduced in the contralateral breasts (0.127° C compared to 0.255° C) and there was a lower probability of detecting a luteal rhythm in these breasts.

Conclusion: These results support the data of Simpson et al and suggest a real difference in the temperature profile of the contralateral breast in breast cancer patients, independent of body temperature. This leads to the hypothesis that a 'high risk' breast may also exhibit this type of temperature profile. The mechanism by which this alteration in local temperature is brought about is unclear but is likely to be a result of local hormonal influences over-riding basal body temperature. Further investigation of these phenomena is warranted.

428 Long-Term Complications Associated with Breast Conservation Surgery and Radiation Therapy.

Meric F, Buchholz TA, Mirza NQ, Vlastos G, Singletary SE, Ross MI, Ames FC, Pollock RE, Feig BW, Kuerer H, Newman LA, Perkins GH, Strom EA, McNeese MD, Hortobagyi GN, Hunt KK. The University of Texas M. D. Anderson Cancer Center, Houston, TX.

Breast conservation surgery and radiation therapy have become the standard of care for early stage breast cancer. We sought to evaluate the long-term complications associated with this approach in the modern radiation era.

Methods: From our breast conservation database of 1398 patients, we selected 294 patients treated with surgery and radiation therapy between January 1990 and December 1992 for the current analysis. All patients were prospectively monitored for treatment-related complications. Median follow-up was 89 months (range, 13 - 126 months).

Results: Significant (Grade 2 or higher) long-term complications were identified in 29 (9.9%) patients and included arm edema in 13 (4.5%), breast skin fibrosis in 12 (4%), fat necrosis in 1 (0.3%), pneumonitis in 2 (0.7%), rib fracture in 1 (0.3%), decreased range of motion in 4 (1.4%), neuropathy in 2 (0.7%) patients. Arm edema was more common when lumpectomy was performed with axillary lymph node dissection (13.6%) v lumpectomy alone (3%), (P=0.05). As expected, the rate of arm edema was higher when surgery was accompanied by irradiation of the lymph nodes (18% v 10%, P=0.07), but did not appear to increase with the number of lymph nodes dissected or the number of pathologically positive lymph nodes. The median weight of patients with arm edema was higher (74 v 67 kg, P=0.01). Skin fibrosis was more frequent in patients who received additional radiation fields (P=0.001), who received a boost (P=0.041), and in those with a larger clinical tumor size (P=0.005).

Conclusions: Breast conservation surgery and radiation therapy are associated with long-term complications in only 9.9% of patients. The majority of complications are related to the axillary component of treatment. Incidence of arm edema is directly related to surgical dissection and slightly higher with nodal irradiation. It is hoped that lower rates of arm edema can be achieved with the utilization of sentinel lymph node biopsy.

429 Breast Conserving Therapy (BCT) in the Elderly: An Appropriate Treatment Choice.

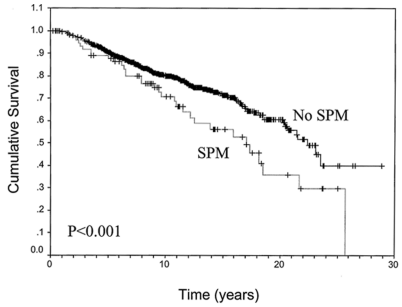
Vlastos G, Mirza NQ, Meric F, Hunt KK, Newman LA, Kuerer HM, Ames FC, Ross MI, Pollock RE, Buchholz TA, Hortobagyi GN, Singletary SE. The University of Texas M. D. Anderson Cancer Center, Houston, TX.

Background: Breast cancer is a major health issue in the United States and the incidence increase with age. However the appropriate loco-regional treatment is controversial in the elderly. Our objective was to evaluate the role of BCT in the management of these patients. **Methods:** Between 1970 and 1994, 1324 consecutive breast cancer patients were treated with BCT (wide local excision and radiation therapy) at a comprehensive cancer center. We identified 184 elderly (>65 years, Medicare definition) women without distant disease. Clinical and pathological features were evaluated and survival calculated using Kaplan- Meier method from the date of initial diagnosis. **Results:** The median age was 70 years (range: 65-88). The stage distribution was as follows: 0 in 12 (6%), I in 108 (59%), II in 62 (34%) and III in 2 (1%). An axillary lymph node dissection was performed in 137 (75%) patients. On pathology review, 142 (77%) patients had invasive ductal +/- *in situ* disease. Axillary lymph nodes were positive in 30 patients (11%). Chemotherapy was given in 10 patients (5%) and hormonal therapy in 63 patients (34%). With a median follow-up of 7 years (range: 0.25-24), 9(5%) patients developed loco-regional recurrence, 11(6%) contralateral breast cancer and 21(12%) distant metastasis. At last follow-up, 116 patients (63%) were alive, 14 died of disease (8%) and 54 (29%) died of other causes. Five- and 10- year disease- specific survival were 96 % and 90 % respectively. **Conclusion:** Breast conserving therapy provides excellent local control and disease-free survival in the elderly. Death from other causes exceeds the risk of mortality from breast cancer.

430 Incidence and Survival Impact of Non-Breast Second Primary Malignancies (SPM) Following Breast Conserving Therapy (BCT).

Mirza NQ, Vlastos G, Meric F, Buchholz TA, Singletary SE, Ames FC, Newman LA, Kuerer HM, Feig BW, Ross MI, Pollock RE, Hortobagyi GN, Hunt KK. The University of Texas M. D. Anderson Cancer Center, Houston, TX.

Our goal was to determine the incidence and survival impact of SPM among BCT patients. **Methods:** Between 1970 and 1994, 1324 consecutive female breast cancer patients were treated with BCT (wide excision and radiation therapy) at a comprehensive cancer center. We identified 130 patients (10%) with SPM; 44% had SPM prior to breast cancer diagnosis and 56% after treatment for breast cancer. Clinical, pathological and outcome parameters were evaluated and survival calculated using Kaplan-Meier method from the date of initial diagnosis. **Results:** The median age was 51 years (range: 21-95) with a median follow-up of 8 years (range: 0.25 - 29). Median tumor size was 1.5 cm. Axillary lymph nodes were positive in 27%. Adjuvant chemotherapy was given to 26% and hormonal therapy to 20% of patients. Contralateral breast cancer occurred in 9%. Patients with SPM after BCT were older ($p<0.001$) and distribution of tumors were; gastrointestinal (22%), gynecological (19%), lung (18%), hematological (8%), thyroid (7%), and others (26%). A higher rate of SPM was not noted in patients that received adjuvant therapy. Overall survival was significantly lower among patients with SPM ($P<0.001$).



Conclusions: Patients with breast cancer are at increased risk for contralateral breast cancer and non-breast second primary malignancies. Patients with SPM have decreased survival and follow-up strategies should include detection methods for other common malignancies.

431 Preliminary Report of Ultrasound-Guided CryoAblation of Breast Tumors Using a 2.4-mm Probe.

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Argon-based cryosurgery has proved successful in the treatment of prostate and liver cancer. We report our experience with the NeoCure™ single-probe cryosurgical system (Sanarus Medical Inc., Pleasanton, CA) for the minimally-invasive treatment of benign and malignant breast disease. To date, our team has treated over 40 patients. These patients can be divided into 3 groups. First, 11 patients (20 breasts) were treated 3 weeks to 6 months prior to elective reduction mammoplasty in order to assess the *in vivo*, histological, cosmetic, and mammographic effects of cryosurgery in normal breast tissue. No serious complications were noted. Both histological destruction and cosmetic results proved to be excellent.

The second group consists of 25 patients with core-biopsy proved fibroadenomas, all under 3 cm in diameter. Two freezes, separated by a thaw cycle were employed in each case, thereby maintaining a total treatment time of less than 30 minutes. All procedures were completed using local anesthetic and minimal intravenous sedation in an office setting. Patients were followed for tumor resolution and cosmesis by clinical exam, ultrasound, and mammogram. Cosmesis was excellent. Typically by 4 months, there was no further mammographic evidence of cryo injury. Ultrasound was much more sensitive at documenting the residual cryo lesion for several months after the procedure. One case of mastitis and 2 hematomas resolved rapidly. While some smaller fibroadenomas may undergo complete resorption by 3 months post procedure, it is more likely to take at least 6 months.

The third group consists of patients whose small breast malignancies were treated with cryoablation 1 to 3 weeks prior to lumpectomy. These specimens were evaluated for extent of cryosurgical destruction of the cancer. Results are promising and suggest that minimally-invasive breast cryoablation should be studied on a larger scale.

432 Uncontrolled Local Disease after Salvage Treatment for Ipsilateral Breast Tumor Recurrence.

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BACKGROUND: Uncontrolled local disease (ULD) following breast conservation constitutes a clinical problem with a major impact on quality of life. The outcome following treatment of ipsilateral breast tumor recurrence (IBTR) and the risk for ULD have been analysed in the present study in order to identify risk factors for ULD.

PATIENTS AND METHODS: In a cohort of 5502 patients treated for invasive breast cancer Stage I and II with breast-conserving surgery 1976-1998 in Stockholm, 307 patients with subsequent IBTR were identified. The majority of patients (n=219) had received postoperative radiotherapy. Median follow-up time was 11(2-23)years. 50/307 patients developed ULD, defined as the appearance of clinically manifest invasive adenocarcinoma in the remaining breast or on the ipsilateral chest wall which could not be eradicated despite surgery, irradiation or/and systemic therapy during at least 3 months. Multivariate linear logistic regression was used in the statistical analysis to identify prognostic factors for ULD.

RESULTS: Five years following the diagnosis of IBTR the cumulative incidence of ULD was 13%. Five independent risk factors for ULD were identified; non-surgical treatment of IBTR, disseminated disease concurrent with IBTR, axillary lymph node metastases (at primary breast conservation), time < 3 years between breast conservation and IBTR, no adjuvant endocrine therapy. Eighty-eight percent of the patients were treated with salvage mastectomy (n=207) or reexcision (n=62). The cumulative incidence at 5 years of ULD following salvage mastectomy respectively salvage reexcision were 10% and 16% compared to 32% among patients treated non-surgically. Following IBTR, the 5-year overall survival among patients with local control was 21% in contrast to 78% among patients with ULD.

CONCLUSION: Uncontrolled local disease is a rare but important outcome measure following breast-conserving surgery. Patients with IBTR, independent of concurrent distant metastases, should be recommended salvage surgery as it provides superior local control compared to salvage systemic therapy.

433 Bilateral Reduction Mammoplasty (BRM) to Improve Breast Conservation Therapy (BCT) Results in Breast Cancer Patients with Macromastia.

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Background: Macromastia has traditionally been considered a contraindication to BCT because the large, ptotic breast is difficult to fix in a reproducible position for irradiation (XRT), and excessive dose inhomogeneity with skin toxicity can result from efforts to ensure adequate therapeutic XRT doses to deeper tissues. On the other hand, unilateral mastectomy in breast cancer patients (pts) with macromastia can result in an unacceptable degree of assymmetry and imbalance. Our goal was to evaluate the feasibility and outcome of BRM to improve results of XRT as a component of BCT for pts with pendulous breasts. **Methods**: Of 153 pts undergoing reduction mammoplasty at the University of Texas M.D. Anderson Cancer Center between 1994 and 1999, 28 were identified as breast cancer pts with macromastia who underwent BRM as a component of BCT. Medical records were reviewed to evaluate outcome, and telephone interviews were conducted regarding pt satisfaction. Median follow-up was 23.8 months. **Results**: Median pt age was 55 years, and 43% presented with a palpable breast mass; median tumor size 1.5 cm. Nearly all pts were described as obese, with a median height of 164 cm and median weight of 79.4 kg. Half (54%) presented with ductal carcinoma in situ or stage I disease. Three pts received neoadjuvant chemotherapy. Median weight of the reduction mammoplasty specimen on the cancerous side was 766 grams, and 645 grams on the contralateral side. Inadequate margin control mandating completion mastectomy occurred in one pt (4%). Six pts underwent lymphatic mapping and the sentinel lymph node was identified in four cases; there were no false negative results. Major postoperative complications occurred in two pts (7%). There were no major post-XRT complications. Pt survey revealed a satisfaction rate of 86%. **Conclusion**: BRM is a reasonable and safe option for reducing XRT-related sequelae in breast cancer pts with macromastia who wish to be treated with BCT.

434 Three-Dimensional Reconstruction of the Whole Breast Duct-Lobular Units Using Computer Graphics; With Special Reference to Ductal Anastomoses between Individual Duct-Lobular Units.

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Introduction: Positive margin mainly arises from intraductal spreading lesions in breast conserving surgery, so understanding construction of duct-lobular units (DLU) is very important. Few studies of detailed anatomy of DLUs have been published because of the difficulty of strict serial microscopic research. We have already revealed figures of intraductal cancer extension through ductal branch anastomoses showing that it can be a risk factor for wide extension of intraductal carcinoma (Cancer 1995; 76: 32-45). In this study, we will report an anatomical feature of ductal anastomoses by three-dimensional reconstruction of a whole breast.

Materials and methods: A whole breast obtained from a 65 year-old woman who underwent subcutaneous mastectomy for intraductal carcinoma was used in this study. Contours of all ducts of serial 2 mm-thick sections were reconstructed by a computer graphic system (TRI; RATOC, Tokyo, Japan).

Results: The whole breast consisted of 16 DLUs. Ductal anastomoses were observed at 11 sites in the breast. Two of 11 were connected adjacent DLUs existed more than 4 cm from the nipple. The remaining 9 existed in the same DLU. In this case, ductal carcinoma was not spreading through these anastomoses.

Conclusion: This is the first report to visualize entire ductal trees of a breast three-dimensionally, revealing that each DLU is not always independent. Ductal anastomoses connecting adjacent ducts were common as were those connecting peripheral duct areas to each other. These are merely results of one woman's breast. However, considered together with our former report, this shows typical breast construction. Ductal anastomoses can be possible routes of wide intraductal spreading which can not be explained by individual independent DLU structure.

435 Positive Margins Following Surgical Excision of Breast Carcinoma: Analysis of Pathologic Correlates.

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Pathologic margin positivity represents a significant source of adverse clinical outcome frustrating efforts to achieve breast conservation for in situ or invasive malignancy. Elucidation of factors associated with positive margin status may clarify and improve local therapy strategies. We therefore retrospectively analyzed the cases of 428 patients who underwent resective procedures for breast carcinoma between 1995 and 1999. Thirty-six pathologic specimens (8.4%) were observed to possess histologically positive margins-defined as malignant cells identified within inked tissue. Twenty-two patients (all female, median age 51.5 years, range 29-70) underwent additional operative procedures, and form the basis for subsequent analysis. Pathologic specimens were reviewed with respect to putative prognostic markers and indices of biologic aggressiveness. Of the 22 re-excision specimens, 14 (64%) contained residual malignacy. Analysis of this material yielded the following results:

	Residual disease (N=14)	No residual disease (N=8)
Histologic type		
Invasive lobular	3	1
Invasive ductal	6	6
In situ ductal	5	1
Multiple sites of margin positivity (% of cases)	71	50
Mean tumor size (cm)	4.3	2.0
Metastatic lymph nodes (mean/case)	1.9	1
p53 mutant gene product (% of cases)	50	25

Though absolute numbers are small, the results demonstate a substantial proportion of patients with residual carcinoma after re-excision have either invasive lobular or in situ ductal histology. Such tumors more frequently demonstrate multiple sites of margin positivity, and are larger than tumors in which no residual disease is present upon re-excision. Expression of the mutant p53 gene product may also be associated with disease persistence following re-excision. These data suggest certain pathologic factors that may portend difficulty in achieving negative resection margins utilizing breast conservation efforts.

436 A Prospective Comparison of 3D Versus 2D Radiotherapy Treatment Planning of Left-Sided Breast Cancer Patients.

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Purpose: To evaluate prospectively if 3D computer assisted radiation therapy (RT) treatment planning results in a reduction of RT dose to the heart compared with conventional 2D planning while not reducing coverage of the tumor region.

Materials and Methods: We conducted a prospective study on RT treatment planning in 32 patients with left-sided breast cancer. Twenty received RT delivered through tangent fields with inclusion of the upper internal mammary nodes (IMN) and 12 patients received tangents only (no IMN). All had CT imaging of the thorax in the treatment position. Two separate simulation procedures were performed using tangent beams with an attempt to block cardiac tissue:

1) Traditional CT-assisted 2D simulation: The desired gantry position and field borders were determined based on the hard copies of the CT images. Fields were designed on the patient in the physical simulator.

2) 3D simulation: The CT data set was placed on a 3D treatment planning system Plan University North Carolina (PLUNC) and tangential beams were designed using virtual simulation.

The plan resulting from the CT-assisted 2D approach was reconstructed on PLUNC. The 3D dose distributions resulting from these 2 simulations were compared with respect to the volume of the heart, IMN, and breast/chestwall receiving the prescribed dose. Patients were treated with the plan which optimized the RT dose.

Results: Overall 72% (23/32) of patients studied appeared to have improved RT plans by 3D over 2D treatment planning. Among the 20 patients in whom IMN were intended to be included in the radiation field, the 3D treatment plan was improved over the 2D plan in 95% of patients. The coverage of the targeted upper IMN, and breast/chestwall was improved with 3D planning in 6 and 8 patients respectively while it reduced incidental heart irradiation in 13 patients. Among 12 patients in whom IMN were not intended to be irradiated, 33% appeared to have a better treatment plan with the full 3D treatment plan by reducing cardiac irradiation.

Conclusions: 3D treatment planning provided better coverage to the targeted treatment areas and less RT to normal tissues particularly when the upper IMN are included in the treatment field. Long term follow-up studies will be necessary to determine if this technology results in better clinical outcomes with respect to local control and normal tissue toxicity.

Work was supported in part by Deparment of Defense Breast Cancer Research DAMD17-98-1-8071.

437 Adjuvant Hypofractionated Conformal Radiation to the Tumor Bed in Selected Post-Menopausal Women with T1 Breast Cancers: A Pilot- Feasibility Study.

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Six weeks of post-operative radiation remain an important deterrent from breast preservation, with patients often selecting mastectomy instead of breast preservation to avoid the inconvenience of six weeks of radiation treatment. Alarming data is also emerging with regard of a sizable proportion of older women who undergo breast preservation surgery but do not receive post-operative radiation. Omitting radiation, however, has resulted in unacceptable local recurrence rates (18-35%); noticeably, in the absence of adjuvant radiation therapy most recurrences occur at the original tumor bed.

Purpose: We explored feasibility of a short course of hypofractionated conformal radiotherapy to the tumor bed as part of a breast preservation protocol in selected post-menopausal breast cancer patients.

Methods: Post-menopausal patients found at segmental mastectomy to have pT1, N0 breast cancers resected with negative margins and no evidence of extensive intraductal component (EIC) were eligible. Tumor bed was imaged at CT in prone position on a dedicated table. The same table and position was used for treatment with a 4MV linear accelerator as previously reported (Jozsef et al, Medical Physics, 2000). PTV consisted of the tumor bed defined at post-mastectomy CT with 1-2 cm margins. A short regimen (five fractions) was selected to be tested in a hypofractionated conformal radiation dose-finding study.

Results: Ten patients were accrued: nine revealed to be treatable by the proposed technique after CT imaging (one patient was excluded because of a very lateral tumor) All patients received five fractions over 2 weeks period. Two patients received 470 cGy X5, two patients 500 cGy x 5, two patients 550 cGy x 5 and 3 patients received 600 cGy X 5. Total dose ranged between 2350-3000 cGy. At 32 months median follow up (24-37 months range) all patients are alive and disease free with excellent cosmesis.

Conclusions: The proposed technique is feasible and should be further studied to assess efficacy when compared to that of six weeks of conventional radiation post-segmental mastectomy.

438 The Evaluation of Myocardial Perfusion after Adjuvant Radiotherapy in Left-Sided Breast Cancer Patients.

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AIM: The aim of the study was to evaluate cardiovascular side-effects of radiotherapy in left-sided breast cancer patients treated with surgery and adjuvant radiotherapy with or without chemotherapy.

MATERIAL AND METHODS: In 58 women with stage I, II and III left-sided breast cancer, the myocardial perfusion scintigraphy were performed before, 3 months after and after at least 1 year after the end of the treatment. Twenty five patients (pts) finished 3 steps of examination. Out of 25 pts, 10 were treated conservatively (BCT), 15 underwent mastectomy. Eleven pts received chemotherapy (FAC, CMF). Pts treated conservatively were irradiated to the left breast (high energy photons, 42.5-50Gy/ 2-2.5 Gy per fraction+ boost), pts after mastectomy were treated to the chest wall (electron beams, 45-46Gy/2-2.25Gyper fraction) and to the lymphatic areas.The median time of observation was 18 months.

RESULTS: In 3 patients new defects in the perfusion were detected in scintigraphy after the treatment, but they were not connected with the area of irradiation (posterior wall). In 2 pts new left ventricular defects were located in the anterior wall. The analysis of the isodose distribution could suggest a connection with irradiation. In both cases near 10% of myocardium was encompassed by the isodose 50% and was related to the area described in scintigraphy.

CONCLUSION: The results suggest, that irradiation can influence myocardial perfusion if about 10% of the heart is encompassed by the isodose 50%. The study requires further investigation.

439 The Assessment of the Toxicity of Adjuvant Postmastectomy Chemoradiotherapy in Breast Cancer Patients.

Niwinska A, Pienkowski T, Miskiewicz Z, Stelmaszczyk P. Breast Cancer Dpt., Memorial Cancer Centre, Warsaw, Poland.

AIM: The aim of the study was to evaluate the myocardial and the pulmonal damage in breast cancer patients treated with mastectomy and adjuvant sequential chemotherapy and radiotherapy (Chth and Rt).

MATERIAL AND METHODS: Forty one women (mean age 45 years) with stage T1-3, N0-2,M0 breast cancer, treated with mastectomy and adjuvant sequential Chth and RT were examined in order to assess the side-effects in the lungs and the heart. The radiotherapy was undertaken in all cases. It included the chest wall (electron beams) and the regional lymph nodes (high energy photon beams) with a specified dose 46-50Gy, administered in daily fractions of 2Gy. All patients underwent chemotherapy: 34 received programs with Adriamycin (ADM), 7- without ADM. The computed tomography of the lungs and the echocardiography were performed before the treatment and after at least 1 year of follow-up. The results were analyzed taking into account the volume of the myocardium involved in the area of irradiation and the dose of ADM.

RESULTS: Heart: 7 of 41 pts had abnormal echocardiogram (ventricular dilatation, abnormal Left Ventricular Ejection Fraction). All seven had received ADM. In 5 of them the echocardiographic defects were asymptomatic and reversible (inhibitors ACE) during the first year of follow-up. In 2 pts moderate toxicity was observed. The analysis of isodose distribution excludes radiotherapy as a factor influencing toxicity, but seems to indicate a connection with ADM. Lung: In 20/41 pts discrete fibrosis in apex and near the chest wall were noted, but these changes were asymptomatic and invisible in the chest X-rays.

CONCLUSIONS: Adjuvant Chth and Rt after mastectomy was well tolerated. Abnormalities in the myocardium, noted in 2 pts, seem to be caused by ADM, but not by Rt.

440 Accuracy of Ultrasound in Localization of Breast Boost Field.

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Purpose/Objective: To prospectively compare diagnostic ultrasound to the "gold standard" of surgical clips for localization of the lumpectomy site for electron boost irradiation.

Materials and Methods: Consecutive breast cancer patients referred following lumpectomy underwent diagnostic ultrasound in radiation treatment position 21 to 100 days post-surgery. All patients had 3-6 surgical clips defining the excision cavity. With the probe tangent to mid-cavity, the site was marked on the skin and depth was measured. Target depth was the deepest aspect of the cavity plus a 1cm deep margin. Treatment fields were prescribed with a clinical 2cm margin on the cavity, and electron energy was chosen to cover the target depth. Surgical clip position was assessed on orthogonal simulator films. Localization was considered: adequate, if all clips were within "field" with 1cm margins and the deepest clip was covered by the 90% isodose; marginal, if clips were within "field" with less than a 1cm margin or the deepest clip was covered by the 80% isodose; and inadequate if any clip was outside the "field" or the deepest clip was not covered by the 80% isodose.

Results: Localizations were performed in 54 breasts (52 women). Participants had a mean age of 61 (SD 11) years, and mean BMI of 28 (SD 5). Most patients had early disease (80% stage 0-I; 4% node positive). Mean tumour diameter was 1.7 (SD 1.7) cm. The mean interval post-surgery was 53 (SD 17) days. The mean ultrasound cavity volume was 24cm3, which correlated moderately (r=0.44, p=.001) with the mean site volume of 38cm3 as measured from clips. The mean boost area was 75 (SD31) cm2, and 43/54 (80%) boosts required 12MeV electrons [9MeV: 4/54 (7%); 16 MeV 7/54 (13%)]. Overall, 35/54 (65%) of localizations were adequate, 15/54 (28%) were marginal and 4/54 (7%) were inadequate. Regression showed that lower patient weight (r=-.37, p=.006) predicted adequacy of localization. Patient age, BMI, separation, bra strap and cup size, tumour and specimen size, location of tumour within the breast and interval from surgery were not independent predictors.

Conclusion: The accuracy rate for clinical localization of the lumpectomy site for boost irradiation ranges from 20-50%. Diagnostic ultrasound may be used to improve the accuracy when surgical clips are not present.

441 Factors Influencing Surgical Choices in Women with Breast Cancer.

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In the absence of medical contraindications, survival after breast conservation therapy (BCT), mastectomy (M), and M with immediate reconstruction (MIR) is equal. Between 1995 and 1998, 587 women with DCIS or early breast cancer with no contraindications to BCT or MIR were seen. Of this group, 85.2% chose BCT, 9.2% M, and 5.6% MIR. We examined demographic factors to see if they differed among groups using Fisher's exact test.

Variable	BCT (n=500)	M (n=54)	MIR (n=33)	p-value	pairwise
Age (mean)	54.01	58.63	48.12	<0.001*	BCT vs M BCT vs MIR MIR vs M
Insurance				0.02*	MIR vs M
Private/PPO	74%	60%	88%		
HMO	7%	6%	6%		
Mcare/Mcaid	19%	34%	6%		
Stage				<0.001*	BCT vs M
0	17%	20%	36%		
1	50%	28%	27%		
2	31%	48%	36%		
3	2%	4%	0%		
Prior Breast Bx				0.02*	BCT vs M
No	80%	67%	66%		BCT vs MIR
Yes	20%	33%	34%		

Marital status and employment approached significance (p=0.06), but family history of cancer was not a predictor of treatment choice. Women undergoing M alone were older and more likely to have stage II carcinoma than those undergoing BCT. Patients undergoing M or MIR were more likely to have had a prior breast biopsy than those choosing BCT. These findings suggest a need for patient education strategies that emphasize the lack of influence of age and prior breast biopsy on the use of BCT. Differences in demographic variables may reflect true variations in patient preference among groups, emphasizing the need to address the spectrum of treatment options with patients.

442 Surgical Morbidity and Patient Satisfaction Following Immediate Breast Reconstruction.

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Immediate breast reconstruction (IBR) is oncologically safe and improves psychological outcome when compared with delayed reconstruction but complications are likely to be higher. We present the surgical morbidities resulting from a consecutive series of 148 women who have undergone IBR following skin sparing mastectomy together with patient satisfaction of the procedure. In our unit pre-operative preparation includes a comprehensive counselling service provided by Specialist Breast Care Nurses. We also encourage contact with volunteers from our patient support group who have undergone similar surgery.

Clinical outcomes were assessed prospectively and patient satisfaction with a structured questionnaire and telephone interview.

The median age was 50 years (range 27-74), 100 patients underwent IBR with a Becker prosthesis, 40 with a latissimus dorsi flap and 8 with a transverse rectus abdominis myocutaneous (TRAM) flap. Median follow-up was 48 months (range 1-96). Overall 35% of patients were node positive (n=52), 39% underwent adjuvant radiotherapy (n=58), 28% underwent adjuvant chemotherapy (n=41). Adjuvant Tamoxifen was taken by 54% of patients (n=80). There were 20 deaths due to breast cancer.

Clinical wound infections occurred in 27 (14 confirmed bacteriologically), 13 prostheses were removed because of infection, and 1 deflated. Capsule formation occurred in 22% (n=32) of whom 15 had received adjuvent radiotherapy (capsulotomy was performed in 20). Thirty-seven patients underwent surgery to improve cosmesis (contra-lateral augmentation or reduction, re-positioning, re-sizing, scar revision, conversion from expander to myocutaneous flap, nipple reconstruction). There were no flap losses. Recipient skin necrosis occurred in 6 patients (4 minor, 2 major - requiring surgical debridement). Local recurrence occurred in 3% (n=4), all treated by further local resection. Regarding patient satisfaction, 88% were pleased with their reconstruction and 92% were happy with pre-operative information given.

Morbidity following breast reconstruction using skin sparing techniques is considerable. In spite of this we find high patient satisfaction with outcome in our unit. This is likely to be due to effective pre-operative counselling which ensures an appreciation by the patients of the complexity of this form of surgery.

443 The Effects of Prolonged HRT Treatment in Normal Post-Menopausal Breast Epithelium.

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Our previous study failed to demonstrate that HRT treatment, oestrogen (E) or oestrogen plus progesterone combined therapy (E+P), significantly increased post-menopausal epithelial proliferation.

Evidence suggests that HRT treatment increases the risk of breast cancer only after five years. To address the possibility that increased proliferation in post-menopausal breast epithelia may occur as a result of prolonged HRT treatment (≥5 years), archival samples of breast tissue, containing normal epithelium, from 229 women who had undergone breast biopsy or therapeutic surgery were obtained. The proliferation rate and levels of the oestrogen-regulated progesterone receptor (PR) were assessed. Tissue was immunocytochemically stained for PR and the proliferation antigen Ki67 and the percentage of labelled cells expressed as a labelling index (LI), per 1000 cells counted.

Results

Patient group	Control (n=121)	HRT<5years	E≥5years	E+P≥5years
Ki67 LI	0.21±0.25	0.2±0.22	0.46±0.73	1.09±0.44
PR LI	4.75±3.46	6.14±9.63	11.91±18.06	10.21±5.11

Proliferation was observed to be significantly greater, than control, in women who had undergone prolonged HRT treatment with E ($p<0.05$) or E+P ($p<0.001$, Kruskal-Wallis). In addition, the increase in proliferation observed in E+P treated women was significantly greater than in E treated women ($p<0.05$). PR LI was significantly greater, than control, in women who had undergone prolonged treatment with E+P ($p<0.05$).

These observations potentially explain the increased risk of breast cancer observed in post-menopausal women undergoing prolonged HRT treatment.

444 WITHDRAWN

445 Hormone Receptor Status and S-Phase in Breast Cancers - Relation to Use of Hormone Replacement Therapy.

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An increasing number of women are using hormone replacement therapy (HRT). In premenopausal women hormone levels in the menstrual cycle influences hormone receptor levels, this has also been demonstrated in breast cancer. Data from surgically postmenopausal cynomolgous macaques has shown that monkeys treated with HRT had lower estrogen receptor (ER) levels than controls without treatment, furthermore animals treated with continous combined HRT (CC) had the highest rate of proliferation in breast tissue. Materials and methods: All 344 women 50-65 years (mean age 57 years) who were treated for breast cancer at Karolinska hospital 1993-97 were included. All data was obtained retrospectively from clinical notes and a questionnaire that all patients are asked to complete. The number of HRT users was 128 (mean age 56) and non-users 198 (mean age 58). Data on HRT were missing in 18 cases. ER was determined by an quantitative immunologic method (EIA) in 237 cases and with immunohistochemistry in 57 cases. S-phase was determined in 179 patients. Results: Among HRT users the ER levels in tumour tissue were lower compared to non users (p=0.05), mean values were 1.2 (SD 1.5) and 1.7 (SD 2.2) respectively. Furthermore in women using CC (n=26) tumor s-phase was higher than in women using cyclic treatment, estrogen only and gestagen only (n=36). Mean S-phase values were 5.7 (0-25) vs. 4.0 (0-39) respectively.

The choice of adjuvant treatment depends on hormone receptor status and s-phase expression. Use of HRT at the time of breast cancer diagnosis could influence these factors and consequently the treatment. Further investigations are needed to explore the importance of our findings.

446 Use of HercepTest in Metastatic Breast Cancer Patients for Assignment to Herceptin: A Cost-Effectiveness Analysis.

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The addition of Herceptin (H) to chemotherapy (C) has been shown to improve response rate, time to progression and overall survival among women with metastatic breast cancer (MBC) whose tumors overexpress the HER-2 protein. Level of HER-2 overexpression has been correlated with poorer underlying disease prognosis, but greater benefit from H. We investigated the incremental cost-effectiveness (CE) of H for different levels of HER-2 expression as determined by HercepTest, an imperfect immunohistochemical (IHC) assay used in clinical practice.

Discounted direct medical costs, discounted life-years (LY), and discounted quality-adjusted life-years (QALY) were estimated with a Markov state-transition model for 4 strategies: C for all MBC patients, H+C only for those with strongly positive (SP) HercepTest result, H+C for those with either weakly positive (WP) or SP result, and H+C for all MBC patients. Transition probabilities were derived from a Phase III clinical trial of Herceptin. Performance characteristics of HercepTest were based on concordance with a gold standard IHC assay. Costs and utilities were derived from published estimates and institutional sources. The model assumed that upon disease progression Herceptin is discontinued and costs and prognosis are equal between treatment arms.

Incremental CE ratios for the base case analysis are presented below, where strategies are listed in order of increasing effectiveness and each is compared with the preceding strategy. Results were sensitive to assumptions about H costs, test accuracy, duration of response and health-state utilities.

Strategy	Δ$/ΔLY	Δ$/ΔQALY
C for all	—	—
H+C for SP	$111,000	$135,000
H+C for SP&WP	$134,000	$171,000
H+C for all	$241,000	$305,000

Use of HercepTest to assign MBC patients to treatment with H yields additional benefits at an additional cost. H-containing strategies could become more cost-effective if fluorescence in situ hybridization (FISH) testing is able to assist in the determination of patients most likely to benefit from H treatment.

447 A Micro-Costing Analysis of Pamidronate/Zoledronic Acid Administration.

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BACKGROUND. Currently, pamidronate (pam) is the only bisphosponate approved for prevention of skeletal complications of metastatic bone disease, secondary to breast cancer, however, newer agents are in development. One such product is zoledronic acid (zol), a 3rd generation bisphosphonate with antiresorptive potency 100 times that of pamidronate. Because of its greater potency, smaller doses enable a shorter infusion time than that required for pam-15 minutes for zol vs. 2-4 hours for pam. The objective of this study was to assess the incremental economic cost advantage of this shorter infusion time, in terms of direct medical and opportunity costs. METHODS: A time and motion study of pamidronate/zol therapy was conducted in conjunction with the zol protocol 10 and 11 clinical trials. Study 10 patients received a two or four hour infusion of zol or pam, depending on random assignment. Protocol 11 patients received a 5-minute infusion of either placebo or zol therapy. Direct medical costs were derived by micro-costing the fixed and variable resources needed to administer these therapies. Drug costs were set at parity for this analysis. Opportunity costs, in terms of additional chairs available each day were calculated by subtracting the chairs required per day for zol administration from those required for pam. RESULTS: A per patient direct cost difference of $-46.21 was estimated for zol, primarily reflecting staffing costs. Using a base case model (10 infusion chairs, 8 bisphosphonate patients/day, 250 days of operation/year), this translates into a direct cost saving of $92,420/year. Using this same base case model, this oncology practice could expect to make an additional 1.8 chemo chairs available per day, or 447.5 chairs per year. CONCLUSIONS: At least 50% of breast cancer patients eventually develop bone involvement and become candidates for bisphosphonate therapy. Such a caseload may tax the resources of the average community cancer center. Newer bisphosphonates that offer shorter infusion times will reduce the direct medical resource utilization necessary for their administration, while freeing additional administration chairs.

448 Inhibition of AP-1 Suppresses the In Vitro and In Vivo Growth of MCF-7 Breast Cancer.

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AP-1 transcription factors play a critical role in signal transduction pathways in many cells. We have investigated the role of AP-1 in controlling proliferating signals in breast cancer cell. We have previously shown that AP-1 complexes are activated by peptide and steroid growth factors in normal and malignant breast cells. In this study, we isolated MCF-7 clones that express a specific inhibitor of AP-1 (a dominant-negative cJun mutant, TAM67) under the control of an inducible promoter using the Tet-Off system. In the presence of doxycyline (Dox), the AP-1 inhibitor was not expressed, and the MCF-7 clones proliferated normally in response to serum stimulation. However, when Dox was withdrawn, the inhibitor was expressed, and serum-induced proliferation was blocked. We next investigated whether the mitogenic response to specific growth factors also requires AP-1. MCF-7 Tet-Off-TAM67 cells were grown in the presence of increasing concentrations of estrogen, IGF-1, and EGF under un-induced and induced conditions. These studies showed that the AP-1 inhibitor completely blocked proliferation in response to the peptide growth factors (IGF-1 and EGF). Inhibition of AP-1 did not completely block estrogen-induced growth, but instead slowed the growth of the MCF-7 cells. To further investigate the effect of AP-1 blockade on in vivo tumor growth, we injected the MCF-7 Tet-Off TAM67 cells into nude mice receiving doxycycline to suppress the expression of the AP-1 inhibitor. After the mice developed tumors, they were randomized to either continue to receive Dox, or to have Dox removed from the drinking water. In mice not receiving Dox, the expression of TAM67 was induced, and tumor growth was inhibited, while the tumors in mice receiving Dox continued to grow. These results demonstrate that AP-1 blockade inhibits the growth of MCF-7 breast cancer cells in vitro and in vivo. These results also suggest that agents that block AP-1 activation would be promising agents for the prevention or treatment of breast cancer.

449 Overexpression of the Parathyroid Hormone-Related Protein Receptor Increases the Mitogenic Responsiveness of MCF-7 Breast Carcinoma Cells.

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Parathyroid hormone-related protein (PTHrP) expression in primary breast cancers is strongly linked with the subsequent development of bone metastases and hypercalcaemia. PTHrP stimulates mitogenesis in several cancer cell lines and is co-expressed along with the PTHrP receptor in the majority of primary breast cancers suggesting that it may stimulate proliferation via autocrine/paracrine mechanisms. Recently we found a marked overexpression of the PTHrP receptor in bone metastases compared to primary breast cancers. To assess the potential consequences of PTHrP receptor overexpression we have generated lines of MCF-7 breast carcinoma cells stably transfected to overexpress either the wild type PTHrP receptor (MCF-7^{WTR}) or a mutant receptor (MCF-7^{T410P}) that exhibits constitutive, ligand-independent activation and characterized their mitogenic responsiveness. The data show that overexpression and/or activation of the PTHrP receptor increases mitogenic responsiveness to PTHrP and also to serum. All effects of PTHrP were mimicked by the cyclic AMP-elevating agent forskolin. In addition cell doubling time was significantly less ($p<0.05$) in MCF-7^{WTR} compared to vector transfected cells.

line	PTHrP-induced cAMP (fold)	Increased ^{3}H-thymidine uptake (%)		Doubling time (h)
		PTHrP	2% serum	
vector	1	0	78±28	25.1±1.2
WT receptor	5.4	37±8	122±28	20.5±0.9
T410P	2.6	24±7	191±28	-

PTHrP also induced a small activation of ERK MAP kinases which was mimicked by forskolin and potentiated (by ≈2 fold) the activation of MAP kinase induced by both EGF and IGF-1.

These results show that increased expression of the PTHrP receptor in breast cancer cells increases their responsiveness to the mitogenic actions of not only PTHrP but also serum growth factors. These effects appear to involve signaling via the cyclic-AMP pathway. The PTHrP receptor may therefore contribute to elevated proliferation in primary or metastatic breast cancer and thus is a potential target for new antagonist drugs.

450 Stromal Insulin-Like Growth Factor 2 Is a Favorable Prognostic Marker for Breast Cancer, Modified by Age, Estrogen Receptor and Mannose 6-Phosphate/Insulin-Like Growth Factor 2 Receptor Status.

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Insulin-like growth factor 2 (IGF2) is a growth and survival factor expressed by stromal cells in invasive breast cancer. Two prognostic studies were conducted to investigate the significance of this finding. In a pilot study of 97 patients, stromal IGF2 expression was associated with a favorable outcome ($p=0.014$). Furthermore, in a model that examined the prognostic power of ER and IGF2 status together, IGF2 status significantly added to the prognostic impact of ER, since IGF2+, ER+ cases had a markedly better prognosis than IGF2-, ER- cases ($p=0.0021$). The favorable influence of IGF2 did not reach statistical significance in an independent data set of 391 patients ($p=0.168$), but did achieve borderline significance in women aged 50 and over ($p=0.051$). In a multivariate analysis, ER and IGF2 were expressed independently. Nonetheless, the prognostic model based on IGF2 and ER status developed in the pilot set was confirmed and was particularly significant in the older age group (ER+/IGF2+ versus ER-/IGF2-, patients 50+ $p=0.0007$, patients <50 $p=0.67$). These exploratory subset analyses therefore suggest that stromal IGF2 expression is characteristic of indolent breast cancer and that the absence of both ER and IGF2 portends a poor prognosis in postmenopausal women. These data also provoke the hypothesis that the contribution of stromal IGF2 to breast cancer pathogenesis is more significant in older patients, perhaps because systemic IGF1 levels decline after menopause. The putative tumor suppressor mannose 6-phosphate/insulin-like growth factor 2 receptor (M6P/IGF2R) was also studied to determine the prognostic implications of the balance set by stromal IGF2 and the IGF2 antagonist properties of epithelial M6P/IGF2R. The favorable influence of IGF2 on prognosis was restricted to M6P/IGF2R- cases ($p=0.022$), suggesting M6P/IGF2R expression attenuates the prognostic significance of stromal IGF2. Although M6P/IGF2R expression status alone was not prognostic ($p=0.292$), loss of M6P/IGF2R expression was also found to have an adverse effect on the prognosis of both IGF2- tumors ($p=0.042$) and ER- tumors ($p=0.026$). These findings indicate that decreased M6P/IGF2R expression is a feature of breast cancer progression, but the prognostic consequences are dependent on the coexpression status of other signal transduction proteins.

451 Growth Factor Regulation of the Forkhead Transcription Factor FKHR in Human Breast Cancer.

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In the absence of growth factors, forkhead transcription factors (FKHR, FKHRL1 and AFX) reside in the nucleus, where to date they have been shown to have two functions: inducing G1 arrest through up regulation of p27kip, and promoting apoptosis. Growth factor activation of the serine/threonine kinase Akt leads to phosphorylation and subsequent nuclear exclusion of the forkhead transcription factors. Different studies have shown this event to be associated with cell cycle re-entry and anti-apoptosis. In this study, we found FKHR was expressed in MCF-7 cells. After treating cells with the erbB2/erbB3 receptor ligand heregulin or insulin-like growth factor I (IGF-I), we found that Akt was phosphorylated, and we observed an electrophoretic mobility retardation of FKHR, which we and others have shown to be due to phosphorylation. Phosphorylation of the Akt consensus site Ser256 of FKHR was confirmed using a phospho-specific antibody. Next, we treated cells with heregulin, purified nuclear and cytoplasmic proteins, then performed FKHR immunoblot. We found that heregulin induced complete nuclear exclusion of FKHR. These results demonstrate that MCF-7 cells express FKHR, and that the growth factors IGF-I and heregulin regulate its function. To further address the role of FKHR in MCF-7 cells, we overexpressed by stable transfection two different green fluorescent protein-FKHR fusion proteins: wild type FKHR (wtFKHR, 3-4 fold overexpression), and an FKHR construct with the 3 serine/threonine Akt consensus sites mutated to alanine (FKHR AAA, 2-3 fold overexpression). The transfected FKHR fusion protein in both clones could be detected by immunoblot due to the slower migration position. Our results to date indicate that p27kip levels were not altered by wtFKHR or FKHR AAA overexpression. In agreement with this finding, IGF-I and serum induced S-phase entry was similar in parental, wtFKHR and FKHR AAA cells. In contrast, flow cytometry data indicate that FKHR transfection increased the number of apoptotic cells. Taken together, these data suggest that growth factors regulate FKHR function in human breast cancer, and this regulation may play a role in cell survival.

452 The HGF/SF Antagonist, NK4, Suppressed HGF/SF-Induced Matrix Adhesion, Invasion and Phosphorylation of Paxillin in Human Cancer Cell Lines.

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Hepatocyte growth factor/scatter factor (HGF/SF) plays a major role in the regulation of migration, cell matrix adhesion, invasion and angiogenesis in cancer, via the phosphorlation of its receptor, the c-met tyrosine kinase. This study examined the role of NK4, a newly discovered HGF/SF antagonist, on the matrix adhesion and invasion of human cancer cells.

Human breast cancer cells MDA MB 231 and MCF7 and prostate cancer cells PC-3 and DU145 were tested. Tumour cell adhesion to the extracellular matrix (ECM), Matrigel was significantly increased by HGF/SF (number of adherent cells 8.9±4.7 with HGF/SF vs 3.2±2.6 in control, $p<0.01$). However, this increase was reduced in the presence of NK4 (4.8±3.1, $p=0.01$). In an *in vitro* invasion assay, NK4 was also found to suppress the invasion induced by HGF/SF (invasion index being 143±33.3 with HGF/SF and 103±21.8 with HGF/SF plus NK4, $p<0.01$). In a real time cellular motion analysis, NK4 was able to significantly reduce the migration of these cells (migration distance over 100 mins being 18.7±3.5 in control, 34.6±10.7 with HGF/SF and 25.5±6.5 in a combination of HGF/SF and NK4). It was further shown that HGF/SF stimulation of the cells resulted in an increase in the degree of phosphorylation of paxillin (immunoprecipitation and Western blotting) and an increase in the immunofluorescent staining of paxillin in the focal adhesion complexes. This effect of HGF/SF on paxillin was similarly antagonised in the presence of NK4. We conclude that HGF/SF stimulates cell-matrix interactions, tumour cell motility and matrix invasion, properties required for metastatic spread. This effect of HGF/SF can be inhibited by NK4, by suppression of phosphorylation of paxillin.

453 Peptide Suppression of Breast Cancer Growth: In Search of Mechanisms by Identification of Cellular Targets.

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Human alpha-fetoprotein (HAFP), a growth promoting fetal protein, contains a cryptic (hidden) growth inhibitory peptide (GIP) motif that is exposed by stress/shock conditions. The GIP has been synthesized, purified, characterized, and tested in various breast tumor growth assays. Presently, we have utilized *in vitro* and *in vivo* mammalian breast cancer models to determine the optimal dose for tumor growth suppression and have uncovered potential cellular targets regarding mode of action. First, a cytostatic growth assay was performed which utilized an ATP-bioluminescence (BL) assay of cell viability. Secondly, GIP was screened in five different human breast cancer (HBC) cell lines which included various GIP doses. GIP was then tested *in vivo* in nude mice transplanted subcutaneously with a human ductal carcinoma and in mice bearing a murine breast-derived sarcoma. Finally, GIP was assayed for growth *in vivo* in a mouse mammary ascites tumor. Results of the ATP-BL assay demonstrated that GIP induced cytostasis in both estrogen-dependent and independent HBC cells at doses ranging from 10^{-12} to 10^{-8}M. The cell culture screen revealed that GIP suppressed growth in all 5 HBC cell lines ranging from 50 - 80% at 10^{-7}M GIP. Finally, *in vivo* studies showed that GIP regressed growth in the ductal carcinoma and ascites tumor, but not in sarcoma cells. Genebank data revealed two major groups of cellular targets for possible modes of action which included receptor/binding proteins and transcription-related proteins. These two categories would encompass agents involving: 1) interruption/impairment of cytoplasmic signaling interactions; and/or 2) uncoupling of G-coupled signal cascades by cell surface receptor blockade. Thus, these data suggest that the GIP might serve as a decoy ligand (agonist) to desensitize cellular signaling cascades.

454 Loss of HER-2-Overexpression during Herceptin®-Therapy in Metastatic Breast Cancer.

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The HER-2-Oncogen encodes for a cell surface receptor with tyrosine kinase activity. Overexpression or amplification of HER-2 occurs in about 20-30% of breast cancer patients. Patients with HER-2-overexpression have an increased risk for metastasis and shortened survival. It has been postulated, that HER-2 is a predictor of poorer outcome following chemotherapy and especially endocrine therapy. Studies have shown a clear benefit from anti-HER-2-therapy with Herceptin® in metastatic breast cancer patients with HER-2-overexpression. There are only few data about the biology of recurrences during Herceptin®-therapy.

We have examined the HER-2-overexpression by immunohistochemistry in the metastatic tissues of five patients during Herceptin®-therapy. In the primary tumors of these five patients overexpression of HER-2 occured (IHC 3+). The antibodies A 0485 (DAKO) and CB 11 (Novacastra) were used for the analysis.

All patients had recieved one or more prior adjuvant or palliative chemotherapies. One of the patients with primary bone metastasis developed a chest wall recurrence after 22 months of Herceptin®-therapy. In another patient with cervical lymph node metastases a supraclavicular lymph node metastasis occured during therapy with Herceptin® after 5 months. A patient with intraperitoneal metastasis developed cutaneous metastases after Herceptin®-therapy for 41 months. All these recurrences where HER-2 negative with IHC and the same antibodies. Another patient with bone metastases developed a new bone metastasis after 27 months of Herceptin®-therapy and the fifth patient with multiple metastases developed a thyroid metastasis after 17 months. In the metastases of the last two patients a 3+ overexpression of HER-2 was found.

In summary only in two of five patients with histological confirmed metastatic tissue, HER-2-overexpression was still present.

The interpretation of these results is still in discussion. The binding sites of the diagnostic and the therapeutic antibodies are different and might not be an appropiate explanation, although steric effects could occur. Dose response relationship to Herceptin® and measurement of serum shed antigen have to be investigated closely in these patients. A selection of HER-2 negative cells during Herceptin®-therapy is possible. No therapeutic consequence is based on these results currently. Further studies are needed to clarify the treatment of recurrent disease after Herceptin®-therapy.

455 Identification of AP-1 Regulated Genes in Human Breast Cancer Cells.

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The AP-1 transcription factor is involved in controlling many cellular processes such as cell proliferation, differentiation, and transformation. In breast cells, the AP-1 transcription factor is activated by growth factors required by these cells, including IGF, EGF, heregulin, and estrogen. We have previously demonstrated that constitutive overexpression of cJun in MCF7 cells results in increased AP-1 activity and estrogen-independent cell growth. In contrast, overexpression of cJun dominant-negative mutant (Tam67) suppresses AP-1 activity and inhibits cell growth. To identify genes that are regulated by AP-1 in breast cells, we isolated MCF7 clones that express cJun or Tam67 under the control of an inducible promoter using the Tet-Off system. We first confirmed that AP-1 activity was modulated by overexpressed cJun or Tam67. Then, using cDNA micro arrays and quantitative RT-PCR, we identified a set of genes that were increased upon cJun induction or decreased upon induction of the AP-1 inhibitor. The expression of several genes involved in growth factor signal transduction was found to be modulated by changes in AP-1 activity. AP-1 modulated genes include genes encoding oncogene products (members of the *ras* and *myc* family of oncogenes), signal transduction kinases (MAP kinases), and growth factor receptors (TNF and IGF receptors). We are currently studying these genes to determine which of the gene products are necessary for growth of breast cancer cells. These critical growth regulating proteins will be promising targets for future agents for the prevention or treatment of breast cancer.

456 Imaging of Primary Tumors in Whole Animals Using Laser-Based Tomography.

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We have developed a model in which the motility, adhesion and cell-cell interactions can be examined in live primary tumors in whole animals. Subcutaneous injection of GFP-expressing cells with graded metastatic potential into the mammary fat pad of female Fischer 344 rats generates primary tumors that fluoresce when GFP excited. Moving cells are imaged in the live rats under anesthesia, with either a laser scanning confocal or a multiphoton microscope. Imaging at high resolution in the non-metastatic and metastatic tumors demonstrates significant differences between the two which account for differences in metastatic potential (see abstract by Segall et al.). Non-metastatic tumors are more fibrous and less necrotic with tumor cells that are highly polarized and less motile. Metastatic tumors are more necrotic with tumor cells that are unpolarized except near blood vessels where they are highly polarized with cell protrusions toward the vessel. Metastatic cells move in groups or streams of cells suggesting a preferred micro-environment for locomotion and probable chemotaxis in vivo. Comparison of imaging with the laser scanning confocal and multiphoton microscopes demonstrates the greater imaging depth of the multiphoton microscope (~10x) in these breast tumors. This allows direct and rapid observations of the blood vessel distribution in primary tumors. Multiphoton imaging also permits visualization of extracellular matrix directly due to its autofluorescence in the near UV allowing the observation of cell adhesion and proteolysis in vivo and its correlation with cell motility. This is the first model that allows direct observations of metastasis in an intact orthotopically grown primary tumor while in the live animal.

457 Analysis of a Metastasis Gene on Chromosome 14q.

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Metastatic breast cancer frequently is a fatal disease in women. Our laboratory has determined that loss of distal chromosome 14q is associated with node-negative disease and slower spread to distant sites. Higher resolution LOH studies have narrowed this phenomenon to a region telomeric of 14q32. Using the smallest region of overlap of the LOH breakpoints, we have identified a 1.6 megabase region spanning the metastasis-related gene. We have collected expressed sequence tagged (EST) cDNA clones that define the region, several of which are very strong candidate genes. These clones have been PCR amplified, purified, and arrayed as probes onto nytran filters in duplicate. We have interrogated these arrayed probes with radiolabeled cDNA from a variety of normal, metastatic, and non-metastatic breast cancer cell lines such as MDA-468, MDA-MB-435A, and MCF-10A. Also, we are interrogating the Clontech Atlas Cancer 1.2 and Human Arrays with RNA isolated from node-positive and node-negative human breast cancers with or without LOH. Differences of expression of the breast-specific subset of these clones in cells and tissues of differing metastatic potential are prioritizing these genes as potential candidate metastasis-related genes. Clones showing increased expression in metastatic cell lines and tissues and decreased expression in non-metastatic cell lines and tissues are then being further characterized using in vitro studies such as quantitative RT-PCR. Ultimately, in vivo studies such as analysis of antisense- and/or sense-expressing candidate genes transfected into the MDA-MB-435 cell line mouse metastasis model will be used to confirm the function of candidate metastasis genes.

458 KGF-Induced Gene Expression in MCF-7 Cells Using cDNA Expression Arrays.

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Breast cancer metastasis is associated with the motility and invasiveness of breast cancer cells. In a previous study we reported the motility enhancement effect of keratinocyte growth factor (KGF) on ER-positive breast cancer cells (Rajah, TT, et al., Breast Cancer Res. Treat 57(1):113, 1999). This study established the KGF motility response and determined that KGF-induced motility was reduced or eliminated by antiestrogen pretreatment. The objective of the present study was to identify genes involved in the KGF motility response in MCF-7 cells. Using cDNA expression assays, we compared the expression of mRNA in control and KGF-treated MCF-7 cells. The Clontech Human Cancer 1.2 and Human 1.2 Atlas Arrays were used permitting the evaluation of over 2000 known genes. Scatter plots and cluster analysis of gene expression (Array Vision software) were used to compare the mRNA expression in KGF treated vs. control cells. It was determined that over 100 genes were up-or down-regulated from 3-100 fold at 1-hr following KGF treatment. We identified 21 up-regulated and 5 down-regulated target genes that are associated with some aspect of tumor progression, proliferation or metastasis. Importantly, the KGF receptor gene was up-regulated over 10 fold. Other up-regulated genes included oncogenes (c-fos and c-jun and c-myc); genes related to tumor promoting growth factors (EGF, EGFR, IGF, HGF, TGF-beta, GRB2, FGF-3, RXR-beta, MIC-1); and established breast cancer biomarkers (c-myc, CSF). It is significant that many of the genes were up-regulated to approximately the same degree on both the Human and the Human Cancer arrays. Concerning the down-regulated genes, it was found that tumor suppressor protein DDC, cell adhesion protein (cadherin), plasminogen activator inhibitor and pleiotrophin (a potential KGF inhibitor) were down-regulated 10-100 fold.

Specific genes and patterns of gene regulation may provide important new information concerning the mechanism of tumor cell motility and serve as biomarkers of tumor progression toward metastasis. (Supported in part by NIH grant # CA 62117 and a grant from the Presbyterian Health Foundation).

459 The Transfection or Induction of Keratin 18 in MDA-MB 231 Breast Cancer Cells Results in Redifferentiation and Strongly Reduced Malignancy of the Clones.

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In retrospective studies on ductal breast cancer we were able to show that a high expression of keratin 18 (K18) in the tumor is correlated with a favorable prognosis for the patient. To get insight into the underlying mechanisms we transfected the human K18 gene into the aggressive cell line MDA-MB 231. In addition, selecting for cellular adhesion in weekly trypsinations we were able to raise a K18 expressing subclone without gene transfer.

Both subclones are characterized by a high expression of K18 in contrast to the parent cells which are virtually devoid.

(i) The morphology of MDA-MB 231 cells in culture is of the dedifferentiated, "malignant" type: spindle shaped, motile, loosely associated. Both subclones are growing in very dense monolayers with epithelial morphology. (ii) The invasiveness in the Boyden chamber is dramatically reduced for both subclones. No metastasis in the nude mouse could be observed. (iii) In both subclones the expression of adhesion proteins is clearly enhanced. The mesenchymal filament protein vimentin is completely replaced by keratins.

In epithelial cells the intermediate filaments of the cytoskeleton are formed by keratins and K18 is a marker of well differentiated mammary lumenal cells. The loss of K18 and its replacement by vimentin is part of a general loss of differentiation along with the malignant transformation. This dedifferentiation seems to be reversible by a re-expression of K18.

460 NK4, a HGF/SF Antagonist, Inhibits Breast Cancer Cell Invasion of Endothelium: Its Role in HGF/SF Induced Changes of Tight Junction Function.

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Permeability of endothelial cells is governed by tight junctions, the most topical structure in the endothelium which create an intercellular barrier and intramembrane diffusion fence. Interaction and penetration of endothelium by cancer cells is an important step in the formation of metastases, indicating that changes in tight junction formation will be an early and key aspect. NK4 is a newly discovered variant of HGF/SF (Hepatocyte Growth Factor/Scatter Factor) that has already been shown by us to be antagonistic to HGF/SF, a cytokine regulating cell motility, migration, proliferation, morphology and angiogenesis.

This study sought to examine the effect of NK4 on (i) HGF/SF induced change in TER (Transendothelial Resistance), (ii) HGF/SF induced change in paracellular permeability and (iii) invasion of endothelium by the breast cancer cell line MDA 123. Co-culture of human umbilical vein endothelial cells (HUVEC) with HGF/SF alone decreased transendothelial resistance over 1 h (from a resistance level of 380 ±8.1 in the control to 204±2.6 with HGF/SF). Addition of NK4 inhibited the HGF/SF induced decrease in TER (to 356±1.5, at p=0.05, n=3). Paracellular permeability of HUVEC's was increased on treatment with HGF/SF 1 h (from a relative fluorescence level of 6775±3.2 in the control to 16375±5.1 with HGF/SF 10 ng/ml). Again, addition of NK4 inhibited the action of HGF/SF (9736±3.0, at p=0.01, n=3. HGF/SF (5 ng/ml) promoted invasion of endothelium by cancer cells (cells invading in control 3±1.2, with addition of HGF/SF 4.6±1.1). This increase in invasion was reduced by addition of NK4 at 100 ng/ml (cells invading were 2.8±1.8, at p=0.01, n=5).

In conclusion, we report that the HGF/SF antagonist NK4 inhibited both breast cancer cell invasion of endothelium and HGF/SF induced changes in tight junction function. NK4 may therefor have a role to play in the control of invasion of the endothelium by breast cancer cells.

461 The Rate-Limiting Step in Metastasis: In Vivo Analysis of Intravasation at the Primary Tumor.

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Determination of the rate-limiting step in tumor cell metastasis is critical for evaluating the cell mechanisms controlling metastasis. Using GFP transfectants of the metastatic MTLn3 and non-metastatic MTC cell lines derived from the rat mammary adenocarcinoma 13762 NF, we have measured tumor cell density in the blood, individual cells in the lungs, and lung metastasis. Correlation of blood burden with lung metastases indicates that entry into the circulation is a rate-limiting step for metastasis for both metastatic and non-metastatic cell lines. Consistent with previous work, cell arrest in the lungs is efficient, while growth of metastases in the lungs is inefficient. To examine cell behavior at the critical step of intravasation, we have used GFP technology to view these cells in time lapse images within a single optical section using a confocal microscope (see abstract by Condeelis et al.). In vivo imaging of the primary tumors of MTLn3 and MTC cells indicates that both metastatic and non-metastatic cells are motile and shoe protrusive activity. However, metastatic cells show greater orientation towards blood vessels, and larger numbers of host cells within the primary tumor, while non-metastatic cells show greater fragmentation. Metastatic cells show chemotactic responses to EGF in vitro, and such responses in vivo may enable metastatic cells to avoid fragmentation and thus enhance their ability to survive entry into the circulation. These results demonstrate that cell-based assays for determination of cell properties in vivo are necessary for dissection of the metastatic process.

462 A Multi-Organ Metastatic Orthotopic Patient ER/PR-Positive Breast Cancer Nude Mouse Model.

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We report here the development of an orthotopic-transplant model of human-patient breast cancer in nude mice that mimics highly metastatic disease. The model was established with primary tumor tissue from a patient with a stage II B, moderately differentiated invasive ductal ER/PR-positive breast carcinoma. Histologically-intact patient tumor tissue was transplanted to the mammary fat pad of 5-week-old, non-estrogen-supplemented nude mice by surgical orthotopic implantation (SOI). The tumor tissue grew extensively and metastasized to numerous organs. Forty-five days after tumor implantation, all animals were sacrificed. At autopsy, 100% of the animals presented with lymph node and lung metastases. Sixty percent of the animals presented with adrenal gland metastases, 30% of the animals presented with heart metastases and 20% of the animals presented with femoral bone metastases. This highly metastatic model of human-patient breast cancer, termed AC3897, closely resembles clinically advanced disease. This model should facilitate development of new therapeutic strategies for treatment of metastatic breast cancer.

463 A New Murine Syngeneic Model of Breast Cancer Metastasis to Bone Expressing PTHrP.

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Bone is a common site of metastasis in advanced breast cancer. Evidence from patient and xenograft (MDA-MB-231) studies show that parathyroid hormone related protein (PTHrP) produced by tumour promotes erosion of bone by osteoclasts, facilitating colonisation of bone by tumour. We have shown that a murine mammary carcinoma cell line (EMT6) expresses mRNA for mouse PTHrP (RT-PCR and Southern blotting). The importance of the intra-cardiac (i.c.) injection route to promote formation of bone lesions is well established.

EMT6 cells (104-2x105) were injected i.c. into 4-5 week old Balb/c mice, which were monitored and terminated at 12-14 days when their clinical condition deteriorated. Lesions in bone were evaluated by X-ray and histology and quantified by image analysis. Bone tumours formed in the distal femora and/or proximal tibia in 54% of mice (13/24) given 1-4x104 cells and in 100% (n=8) of mice given 1-2x105 cells. Lesions were easy to identify and occupied up to a third of the total area in affected bones. The cross sectional area of bone in mid-line longitudinal sections was reduced to 39% (median value, IQ range 32.6-46.9) in tumour bearing bones compared with 45% (median value, IQ range 39.9-51.8) in bones from control animals or that did not develop tumour lesions. This is evidence for significant bone loss associated with tumour infiltration (Mann Whitney p=0.044). The efficacy of anti-PTHrP therapy is currently being evaluated in this model.

In conclusion, we have established a new syngeneic mouse model of breast cancer metastasis to bone using EMT6 cells that also express PTHrP. Since Balb/c mice have an intact immune system, the syngeneic EMT6 model should prove a useful adjunct to established in-vivo xenograft models in which to investigate the role of PTHrP in bone metastasis and develop anti-PTHrP therapies.

464 Palpation Abets Breast Carcinoma Dissemination in the Setting of Lymphovascular Invasion.

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Palpation of breast cancer invariably occurs during breast self-examination, physical examination, mammography and surgical resection. Historically there have been inferences that such palpation may abet tumor dissemination but these innuendos have never been tested scientifically. The present study addressed this question from a mechanistic standpoint using three different human tumoral xenograft models: a non-metastatic xenograft, a highly metastatic xenograft which metastasizes as single cells and a unique xenograft which exhibits local lymphovascular emboli (lymphovascular invasion). Palpation was carried out every third day over two weeks when the xenografts were between 1.0-1.5 cm in size. Palpation increased the intra-tumoral pressure of each xenograft similarly by 200 percent (10→30 mm Hg) but dramatically increased the number of pulmonary lymphovascular embolic metastases by 100 fold only in the xenograft exhibiting lymphovascular invasion (LVI). The mechanism of this effect was through an immediate post-palpation release of circulating tumor cells detected by human cytokeratin 19 RT-PCR of murine blood and not through a release of autocrine or paracrine growth factors such as IGF-I, IGF-II, TGF-α and TGF-β or angiogenic factors such as VEGF or bFGF. In contrast, palpation did not abet dissemination of the non-metastatic xenograft or the highly metastatic xenograft which metastasizes as single cells. Therefore palpation promotes only a very specific step of the metastatic process, embolic dissemination and not other steps such as invasion, intravasation and extravasation. Breast cancers exhibiting exaggerated LVI might be particularly susceptible to palpation-abetted dissemination.

465 Gemcitabine Completely Suppressed the Stimulating Effects of G-CSF on Clonal Proliferation and Migration of Cancer Cell Lines *In Vitro*.

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Human recombinant G-CSF (hrG-CSF) is routinely used for mobilization of stem cells from peripheral blood(PBSC). Transplantation of PBSC for rescue of bone marrow function after high-dose chemotherapy (HDC) is widely used in breast cancer tumors. Evidence is accumulated that the contamination of hematopoietic grafts with tumor cells may contribute to breast cancer recurrence. The aim of our studies was to investigate both the effect of hrG-CSF on clonal proliferation and migration of breast cancer cells and the ability of Gemcitabine to supress these processes. Gemcitabine (GEM) is a novel pyrimidine antimetabolite in the cancer therapy. Our data showed that at peak plasma concentrations (10 - 20 ng) hrG-CSF significantly stimulated the clonal growth of breast cancer cell lines, MCF7 and MDA-MB-231 *in vitro*. The stimulation of migration was observed only MDA-MB-231, but not MCF7 cells. GEM was a very potent inhibitor of clonal proliferation of breast cancer cells with ED50 at 10^{-12} M. Interestingly, the GEM completely supressed the stimulating effects of hrG-CSF on clonal proliferation and migration of cancer cell lines *in vitro*.

In conclusion, the combination of hrG-CSF and GEM should be subject of further investigation, in order to improve for complete eradication of tumor cells in PBSC of breast cancer patients.

466 The Role of Caveolin-1 Gene Expression in Progression and Metastasis of Human Breast Cancer.

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Caveolin-1 potentiated ER-mediated signal transduction. Based on this, it has been suggested that the caveolin-1 expression may help prevent turmorigenesis and metastasis by promoting estrogen transcription in breast cancer cells. Despite these lines of evidence for caveolin-1 gene as a tumor suppressor, recent studies have reported that the expression of caveolin-1 gene did increase in metastatic clones of breast and prostate cancer. In addition, this gene is closely correlated with hormone independence in prostate cancer. Because of controversies in the role of caveolin-1 in tumor behavior, we investigated the role of caveolin-1 gene in breast cancer. In MCF-7 cells, the expression of caveolin-1 gene was up-regulated by estradiol whereas it was down-regulated by tamoxifen. Paraffin sections from 61 breast tumor specimens were analyzed for expression of caveolin-1 gene by immunohistochemistry. Among 61 cases, 38 cases displayed extensive caveolin-1 staining comparable to that of adjacent normal epithelial cells, whereas 23 cases showed either significantly decreased or no caveolin-1 staining and 9 cases had a focal staining pattern. Caveolin-1 gene was more frequently overexpressed in ER (-) breast tumor (P=0.001). Breast cancers with ER (-) and caveolin-1 (+) had high frequency of recurrence within 4 years (P=0.027). These data indicate a possible interaction between up-regulation of caveolin-1 gene and metastasis (recurrence) in breast cancer. We are currently performing an in vivo experiments (foot fat pad injection of tumor cells into nude mice) using MCF-7 cells transfected with caveolin-1 gene to determine its roles in metastasis.

467 Analysis of Type 1 and Type 2 T Cells in Breast Cancer by Intracellular Cytokine Staining and Flow Cytometry.

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Helper T cells (Th) and cytotoxic T cells (Tc) can be categorized as Th1 and Th2 (Tc1 and Tc2) on the basis of the cytokines they produce. For example, Th1 cells produce interleukin-2 (IL-2), interferon-g (IFN-g), and tumor necrosis factor (TNF), while Th2 cells produce IL-4, IL-6 and IL-10. These cells play important immunoregulatory roles and recent studies have suggested that an imbalance of the normal ratio of type 1 and type 2 T cells (eg. a decrease in type 1 cells and/or an increase in type 2 cells) may result in impaired cell-mediated immunity in cancer patients.

In this study, we assessed the intracellular cytokine profiles of CD4+ Th cells and CD8+ Tc cells in the peripheral blood of women with breast cancer. Peripheral blood samples were obtained from patients with breast cancer prior to their first surgical resection, as well as from healthy volunteers. Cells were analyzed by 4-color flow cytometry for surface markers (CD3, CD4, CD8, and CD69) and for intracellular cytokines (IL-2, IFN-g, TNF, and IL-4). The percentage of Th1 and Tc1 cells, as measured by IFN-g or TNF production, were significantly lower in the patients with breast cancer compared to the healthy voluteers. Interestingly, this depressed type 1 response was comparable across all stages of disease (I-IV). However, those patients with no positive lymph nodes had better type 1 responses (albeit still lower than the control group) than those patients with positive nodes. Data will also be presented with respect to type 2 responses in these patients.

Our results indicate an alteration in the balance of type 1 and type 2 T cells in patients with breast cancer. These studies are ongoing and may find application in the diagnosis and therapeutic monitoring of patients with breast cancer.

468 Cellular Immune Responses to Immunodominant HER-2/neu Helper Peptides in Patients with Ductal Carcinoma In Situ (DCIS).

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Since the advent of mammography the diagnosis of DCIS has increased over 500%. DCIS is a preinvasive breast tumor which carries an increased risk of recurrence and the possibility of developing into invasive cancer depending on size, grade, and the presence of comedo necrosis. Over 80% of DCIS demonstrating comedo necrosis express the HER-2/neu protooncogene. Hence, development of an HER-2 based vaccine strategy might be useful in the treatment/prevention of this disease. To determine whether patients with DCIS were capable of mounting an immune response to HER-2 derived HLA class II restricted helper peptides G89 (SPYVSRLLGICLT) and G90 (IKWMALESILRRR), peripheral blood lymphocytes (PBL) were incubated with peptides or no peptide (control) for five days at 37°C. Cells were then pulsed with ^{3}H-Thymidine and cpm for PBL stimulated with peptide were compared to cpm from unstimulated PBL. Mean ± S.D. cpm which were ≥ 3 S.D. above that for NP control were considered significant at $p \leq 0.05$. Using these criteria 7/11 patients responded to dominant peptide G89 compared to 4/11 patients to peptide G90. Stimulation indices (S.I.=cpm with peptide divided by cpm NP) were higher for responding patients (2.1 ±0.19) than for nonresponders (1.2±0.22; $p<0.0001$). The percentage of CD4 positive T-cell precursors correlated with the degree of proliferative response to peptides. A comparison of the degree of immune response to HER-2/neu status as measured by FISH is in progress. Supported by DAMD grant 17-94-J-4313 and core grant CA16672.

469 Elevated Serum Levels of Soluble ICAM-1 Observed in Patients with Breast Cancer Do Not Interfere with Anti-Her-2/neu Antibody Mediated Cytotoxicity.

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Anti-Her-2/neu antibodies have considerable activity in patients with Her-2/neu overexpressing breast cancer. However, even in patients with Her-2/neu overexpressing tumors response rates are only 16% suggesting that additional factors influence the activity of this therapeutic regimen. In previous experiments we demonstrated a decreased expression of intercellular adhesion molecule 1 (ICAM-1) on monocytes and tumor cells in breast cancer patients resulting in reduced tumor cell lysis. Since several reports described increased levels of soluble ICAM-1 (sICAM-1) in the sera of breast cancer patients and found sICAM-1 to inhibit natural killer and lymphokine activated killer cell activity we were interested to elucidate the effects of sICAM-1 upon anti-Her-2/neu antibody mediated cytotoxicity (ADCC).

Peripheral blood mononuclear cells from 14 chemonaive patients with metastatic breast cancer and 7 matched controls were isolated by a buoyant denstiny gradient. Subsequently, cells were coincubated with Cr^{51} labelled Her-2/neu overexpressing SKBR-3 and Her-2/neu negative HTB-132 cells in the presence or absence of sICAM-1 at concentrations found in patients with early (250ng/ml) and advanced breast cancer (500ng/ml), respectively. Cytolysis was calculated according to Cr^{51}-release measured by a γ-counter.

No significant difference was observed between patients and controls concerning unspecific cytotoxicity against SKBR-3 and HTB-132 cells(lysis rates: 7,96±4,16% and 8,95±5,26% in SKBR-3 cells versus 2,50±1,06% and 1,93±2,04% in HTB-132 cells). ADCC was significantly higher in SKBR-3 cells (34,84±13,08% in patients, 35,55±9,28% in controls), but not in Her-2/neu negative HTB-132 cells (2,48±1,68% in patients and 1,86±1,47% in controls). In both cell lines, unspecific as well as antibody-mediated cytotoxicity remained unchanged in patients and controls by the addition of sICAM-1: At concentrations of sICAM-1 observed in patients with metastastic breast cancer lysis rates in SKBR-3 cells were 34,51±12,47% and 38,21±10,83% in patients and controls, respectively, in HTB-132 cells 2,33±2,60% and 2,48±2,22% in patients and controls, respectively.

Our results demonstrate that sICAM-1 levels observed in the sera of patients with breast cancer do not interfere with anti-Her-2/neu antibody mediated ADCC.

470 Expression of HER2 and HER1 (EGFR) in the Myocardium as a Cause for the Cardiotoxicity of Trastuzumab (Herceptin).

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The combination of the FDA approved HER2-antibody trastuzumab (Herceptin) with anthracyclines in the therapy of metastatic breast cancer proved to be very effective. However, this regimen is limited by a dramatic increase in cardiotoxicity. Since HER2 plays an essential role in cardiac hypertrophy as well as in embryonic cardiogenesis, there might be a putative interaction of trastuzumab with HER2 expressing myocardial cells. HER2 is one of four distinct members of the EGF-receptor family besides HER1, HER3 and HER4. Considering the different possible dimerisation patterns between these receptors, we also determined the coexpression of HER1.

The expression of HER2 and HER1 was analyzed in three groups with a total of 85 specimens: I. heart tissue of 20 patients with a history of anthracycline therapy for breast cancer; II. heart tissue of 15 breast cancer patients without anthracycline treatment; III. biopsies of 50 patients with decreased left ventricular function. The histological alterations ranged from acute and chronic myocarditis to different degrees of myocardial hypertrophy.

HER2- and HER1-expression was analyzed immunohistochemically using the HercepTest or the primary antibody A 0485 of DAKO and Ab-10 of NeoMarkers respectively. HER2 gene amplification was analyzed by fluorescence in situ hybridization (FISH) using the Inform-Kit of Ventana.

In all specimens no HER2 gene amplification could be detected by FISH. The immunostaining of HER2 was negative in most cases but in a few biopsies a light, discontinuous membrane staining was notable. In all specimens tested no HER1 staining could be observed. The activation of residual HER2 via heterodimerisation with HER1 can be excluded but a possible role of HER3 and HER4 remains to be clarified.

471 Defective Expression of Adhesion Molecule CD54, CD80 and CD86 Resulting in a Defective Antigen-Induced T-Cell Proliferation in Breast Cancer.

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Previous experiments from our laboratory have shown that the expression of CD54, the production of tnf-a by monocytes (M0) and appropriate function of lymphocyte subpopulations were defective in BC. CD28 and CD152 costimulatory molecules have been shown to play a decisive role in the initiation of an appropriate immune response, which is the ligand for CD80 and CD86 antigens present on activated antigen presenting cells (APC). We have investigated the expression of CD54, CD80 and CD86 expression on M0 derived from patients with BC and its regulation by TNF-a production by these cells and the resulting consequences for antigen presentation.It was found that M0 derived from patients with BC had a significantly decreased expression of CD54 ($p<0.0001$) and a decrease in production of TNF-a ($p=0.03$) which would able to correct the former deficiency and, subsequently, a significantly decreased ability to present the antigen tetanus toxoid ($p<0.0001$), as compared to M0 derived from HCG.In order to investigate APC-associated CD80 and CD86 interaction, the presence of the latter antigens on M0 were analysis. M0 derived from patients with EBC were found to express CD80 as well as CD 86 at significantly lower levels than those observed on M0 derived from HCG(CD 80: $p=0.009$; CD 86: $p=0.002$). A significantly decreased proliferation of T-cells in response to TT was found in patients with EBC, as compared to HCG ($p<0.0001$). We incubated M0 and T cells from HCG in presence of TT and anti CD80 antibody. In these experiments, anti-CD80 antibody was able to inhibit T cell proliferation significantly in a dose dependent manner (50ng anti CD80-antibody: $p=0,005$; 100ng anti CD80-antibody: $p<0.0001$).It is concluded that M0 derived from patients with BC had a simultaneous defect in CD54, CD80 and CD86 expression, TNF-a production and, finally, antigen presentation. It is speculated, that the described deficiencies in costimulatory molecule expression and the resulting defect in antigen presentation in EBC might contribute to the state of tolerance of the immune system to the presence of malignant cells in this disorder.

472 First Identification of Tumor Specific Antigen Mage-b3 in Two Transgenic Breast Tumor Models Driven by c-myc and v-Ha-ras Genes.

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Expression of Tumor Associated Antigens (TAA) such as MAGE, BAGE, GAGE, NY-ESO-1 have been identified in various human tumors. Because the expression of these TAA is restricted to the tumor cells only, they are attractive targets for immunotherapies against cancer. TAA are able to activate Cytotoxic T Lymphocytes (CTL) that may lead to destruction/elimination of the tumor. In 12% of human breast tumors MAGE-3 gene expression have been detected. However, until today there are no suitable animal breast tumor models expressing this antigen available for the development of a breast cancer vaccine.

The aim of this study is to develop suitable breast cancer model by screening mammary tumors of transgenic mice for the expression of mouse TAA Mage b1, b2, b3, homologous to the human MAGE genes.

In the study presented here, Mage-b3 mRNA products were identified in mammary tumors of MMTV-v-Ha-ras and MMTV-c-myc transgenic mice. Mouse Mage-b3 is homologous with human MAGE-3. We sequenced and compared Mage-b3 gene from both types of tumors and testis with the published DNA sequence of Mage-b3 derived from kidney. Nucleotide differences were found in the cDNA of Mage-b3 in comparison to the published DNA sequence. These differences may lead to new peptides and therefore may change their ability to activate CTL against tumors. Detailed sequence data of Mage-b3 from all specimens and the consequences of such changes for the peptide CTL epitopes and vaccine development will be presented.

473 Tumor/Bone Marrow Dynamic Reciprocity: Intimate Interactions with Endothelium May Confer Selective Advantage to Breast Cancer Cells.

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The presence of micrometastatic cells in the bone marrow (BM) has been correlated with worse prognosis in patients with early stage breast cancer (BrC). BrC cell survival in the BM may be associated with the establishment of interactions between the tumor cells and the BM microenvironment. As de novo angiogenesis has been associated with the development of BM malignancies, we sought to determine the nature and functional properties of the interactions between BrC cells and human BM endothelium. Co-culture of BrC cell lines and BM endothelium in a gel rich in extracellular matrix components (Matrigel) resulted in the formation of multicellular network structures, in which BrC cells associated with BM endothelium capillary-like structures. This association was also evident when BrC cells were added to previously-organized BM endothelium, showing that tumor cells actively migrate through the gel to adhere to the BM endothelium. These interactions were observed with both a BrC cell line that organize as network structures (MCF7) and tumor cells that form independent clusters (MDA-MB231). Interestingly, non-malignant mammary epithelial cells (MEC) also adhered to BM endothelium, suggesting that this may be a general property of the mammary epithelium rather than of their malignant phenotype. The association between MCF7 cells and the BM endothelium remained stable for up to 10 days. In contrast, the structures involving MEC and BM endothelium, as well as those formed by MCF7 cells or BM endothelium alone, disintegrated after 4 days. In conclusion, these studies demonstrate that BrC cells establish intimate interactions with organized BM endothelium. These interactions result in the formation of more stable heterotypic structures when malignant, but not normal, mammary epithelial cells are involved, suggesting that a BM endothelium: BrC cell dynamic reciprocity may exist and result in a selective advantage for the tumor.The reciprocal interaction between BM endothelium and breast epithelium may represent one of the survival strategies of metastatic cancer in the BM microenvironment, providing a potential target in the management of BrC.

474 ATP and Metastatic Breast Cancer: Prognostic and Therapeutic Implications?

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Adenosine 5'-triphosphate (ATP) plays a critical role in cellular energy production and metabolism. Extracellular ATP has been shown to interact with both purinergic P1 and P2 receptors, and interactions with the P2 receptor seem to be particularly physiologically relevant. Functionally active P2 receptors have been demonstrated in-vitro on several breast cancer cell lines. Our group, and others, have shown that in-vitro, extracellular ATP induces a significant inhibition of tumor cell growth and proliferation. Induction of apotosis has been demonstrated in these in-vitro systems as well. We have further shown a marked inhibition in growth of human breast cancer xenografts in a double knockout nude mouse model our group has previously developed, in CFTR (cystic fibrosis transmembrane conductance regulator) homozygous mice, in which we have further shown elevated mean blood [ATP], [ATP]/RBC (red blood cell), and plasma [ATP] (Nature Medicine 1996; 2:593-596). Reduced [ATP] has been implicated as well in cancer cachexia, and clinical trials of ATP therapy have suggested potential benefits in cancer cachexia. We now demonstrate that in a small number of patients (pts) with metastatic breast cancer, whole blood [ATP] is significantly decreased, as is the ATP RBC efflux rate, and plasma [ATP]. Data on an expanded cohort of pts will be presented. The table below summarizes our findings comparing intra- and extracellular [ATP] and total ATP/RBC pool in 3 pts vs. age-matched controls (cts).

	Plasma ATP*10^{20} pool*10^{17} ATP(Moles/RBC)	RBC ATP Release Rate (Moles/RBC/min)	Total Blood ATP (Moles/RBC)
pts	1.15-1.67*	0.05-0.49*	12.68-15.60*
cts	3.22-5.64*	0.88-2.16*	18.40-22.90*

*all statistically significant, P<0.0001.

These data further support the potential therapeutic role of ATP, both as a therapy for cancer cachexia, and potentially as a specific cancer therapy for pts with breast cancer. We are currently initiating a phase I clinical trial of ATP to further evaluate these hypotheses.

475 Epithelial and Stromal Clonality of Fibroadenomas and Phyllodes Tumors of the Breast.

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Fibroadenoma and phyllodes tumor are both bifasic tumors. Although epithelial malignancy arising within fibroadenoma has been described, fibroadenoma is considered a benign tumor. Phyllodes tumors are of unpredictable behavior and can grow locally destructive and can even metastasize. A relation between both tumors has been suggested. Goal of our research was to study clonality in epithelium and stroma of fibroadenomas and phyllodes tumors, to test our hypothesis that fibroadenoma may progress in epithelial direction towards carcinoma in situ, and in stromal direction towards phyllodes tumor.

Normal tissue, stroma and epithelium were microdissected from paraffin embedded fibroadenomas and phyllodes tumors under an inverted microscope after laser microdissection. After digestion with methylation sensitive restriction enzyme HpaII and nested PCR amplification of a human androgen receptor sequence with a polymorphic repeat, the product was analyzed on a sequence analyzer. Amplification (AR) and clonality ratios (CR) were calculated. A CR below 0.4 was considered as evidence for monoclonality. All assays were performed in duplicate with consistent results.

So far, twenty of 22 women analyzed were heterozygous for the androgen receptor gene. Both stromal and epithelial components of the 10 fibroadenomas were polyclonal. Epithelial monoclonality was detected in 5 fibroadenomas harboring carcinoma in situ. In two fibroadenomas with an area of phyllodes tumor like stroma, monoclonality was detected in this area, whereas the stroma outside this area was of polyclonal nature. Stroma of most phyllodes tumors was monoclonal and epithelium was polyclonal. However, in two phyllodes tumors epithelium was found to be monoclonal and stroma in two other phyllodes tumors was polyclonal.

Monoclonal epithelium found in phyllodes tumors may indicate that at least in some tumors also the epithelium is neoplastic. The finding of monoclonal stromal areas in two fibroadenomas indicates that fibroadenoma may progress in stromal direction to phyllodes tumor. Therefore, it seems that fibroadenomas have the capability to progress in both epithelial and stromal directions. In addition, the polyclonal stroma of histologic phyllodes tumors may indicate a spectrum between both tumors.

476 Localization of uPA-PAI1 and uPA-PAI2 Complexes in Early Breast Cancer. Correlation with Other Molecular and Biological Parameters.

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In order to investigate the role of uPA and its interaction with its natural inhibitors, PAI1 and PAI2, in early (pT1) breast cancer, we studied 189 samples by means of immunohistochemistry (streptavidin-biotin-peroxidase system), using the Chemicon AB776 polyclonal antibody, which reacts with uPA-PA-1 and uPA-PAI-2 complexes. In addition, CD44std, Ki67, c-erb-B2, p53, ER and PR expression was studied on the same tissue samples by the same method. The obtained results were correlated with nodal invasion, with each other, and with classical pathologic features such as histologic and nuclear grade by means of the Spearman test for nonparametric variables.

Results: The immunohistochemical reaction with uPA-PAI-1 and uPA-PAI-2 complexes was cytoplasmic and localized inside the tumor cells, with no or minimal reaction in stromal cells. uPA-positivity detected by this method correlated significantly with ER expression ($p = 0.031$), PR expression ($p = 0.030$), favorable nuclear grade ($p = 0.0087$) and marginally with a low proliferation rate ($p = 0.088$), which is the opposite of the results reported by most other groups when studying either free uPA or uPA bound to its membrane receptor (uPAr) in similar tumors.

Conclusion: From our results we conclude that uPA-PAI-1 and uPA-PAI-2 complexes are formed inside the tumor cells for the purpose of inactivating free or uPAr-bound uPA, which explains why our findings are symmetrical to those obtained when studying these latter forms. A model incorporating our data and the present knowledge on the uPA-uPAr-PAI chain is proposed.

477 The Antiproliferative Effect of $1,25(OH)_2D_3$ on HC11 Mammary Cells is Not Associated to Induction of TGFβ and $p21^{WAF1/CIP1}$ or Inhibition of *c-myc* Expression.

Folgueira MAAK, Katayama MLH, Snitcovsky IML, Brentani MM.

The cell line HC11 with both *p53* alleles mutated, originated from midpregnant BALB/c mice mammary gland, have retained features of mammary epithelium cells including the capacity of differentiation. After transfection of HC11 with the oncogene Ha-*ras*, the cells assumed transformed properties. Only the parental lineage, which express higher vitamin D receptor (VDR) content than HC11 *ras*, is growth inhibited by the hormone, being arrested in GO/G1 phase without evidence of apoptosis or differentiation. We have attempted to relate growth inhibition to effects on the expression of genes which are potentially modulated by $1,25(OH)_2D_3$ and are linked to control of cell proliferation: *c-myc*, transforming growth factor beta (TGFβ1) and the cyclin-dependent kinase inhibitor $p21^{WAF1/CIP1}$. We have observed that HC11 cells on log growth phase exhibited high *c-myc* and *max* levels (mRNA and protein) which were maintained as cells reached confluence. Little variations on *c-myc* and *max* expression were verified upon $1,25(OH)_2D_3$ treatment. HC11 cells did not express TGFβ1 and $p21^{WAF1/CIP1}$ mRNAs and exposure to $1,25(OH)_2D_3$ have not induced the transcription of these genes. HC11 cells also lack CDK tyrosine phosfatase cdc25A mRNA expression which might be repressed by TGFβ1 and so show no cdc25A response to $1,25(OH)_2D_3$. In addition, only a small fraction of cells displayed the type II TGF-β receptor (TβRII) determined by flowcytometry, suggesting that HC11 normal cells have non functional TGFβ1 pathways . HC11 *ras* cells, presented high *c-myc* and *max* levels, expressed TGFβ1, $p21^{WAF1/CIP1}$ and cdc25A mRNAs, which were not induced upon hormone exposure. TβRII was detected in a small percentage of cells, in resemblance to the parental lineage. Thus *c-myc*, TGFβ1 and $p21^{AF1/CIP1}$ seem not to be mediators of $1,25(OH)_2D_3$ antiproliferative effects on the HC11 mammary cells.

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478 Heparan Sulfate Proteoglycan Expression in Breast Carcinomas and Its Impact on FGF-2 Signaling.

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Fibroblast growth factor-2 (FGF-2) is a mitogen for many cell types, and some investigators report stimulation of breast carcinoma cell proliferation by this growth factor. Binding of FGF-2 to its receptor tyrosine kinases (RTK) and cellular signaling are modulated by heparan sulfate proteoglycans (HSPGs) via their heparan sulfate (HS) side chains. The role of these molecules in breast carcinoma growth control is currently unknown. The goal of this project was to investigate HSPG alterations in breast carcinomas compared to normal mammary gland.

A total of 30 infiltrating breast carcinomas were examined. The ability of HSPGs to promote FGF-2 signaling complex assembly was tested by using FGF-2 ligand and a soluble RTK fusion protein (FR1-AP) as binding probes. HSPG core protein expression was measured by immunohistochemical detection of syndecan-1, syndecan-4 and glypican-1.

In contrast to normal gland epithelium, carcinoma cell HSPGs show increased FGF-2 binding and furthermore the HSPG/FGF-2 complexes immobilize soluble FR1-AP fusion protein, indicating that these HSPGs promote FGF-2 signaling. Surprisingly, no single HSPG core protein co-localizes with this binding activity.

Syndecan-1 is uniformly present in normal gland epithelium, but is heterogeneously expressed in carcinomas. Syndecan-4 is highly expressed in normal gland epithelium, but reduced or lost in most carcinomas examined. It is lower in the infiltrating than in the in situ component, if both co-exist. No dramatic changes in glypican-1 expression are observed.

Loss of syndecan-4 may convey an infiltrating, migratory phenotype to the carcinoma cells. The increased ability of carcinoma HSPG to promote FGF-2 signaling complex assembly is likely due to structural abnormalities of the HS chains rather than altered core protein expression and may contribute to accelerated proliferation.

479 The Growth Rate of Self-Detected Breast Cancers Follows a Similar (Power) Law to Mammography-Detected Cancers.

Shochat E, Cameron DA.

Breast cancer is notorious for its heterogeneity and unpredictable behaviour among patients. Recently the growth rate of screening mammography detected cancers was shown to follow a power law (1). This law indicates unbound growth but with a slowing doubling time as tumours get larger. The substantial decline in the growing tumor growth fraction was shown to effect the chemotherapy sensitivity of primary and metastatic cancers (2). As the screen detected cancers represent only a small fraction of the total tumor population, and the majority of primary breast cancers present symptomatically, there is some concern that these two way of diagnosis may represent two different biological entities. Therefore, we have mathematically analysed a distribution of the pathological size of 250 self detected breast cancers (presenting to a single center in 1988) to derive the most appropriate growth function (i.e. power law, exponential, logistic, Gompertz) which will describe this population. By accounting for the psychophysical process that underlie the tumor detection, a probability model of tumor detection containing an implicit form of the growth function was formulated. Comparing the empirical distribution to the model generated theoretical tumor size distributions indicated that the growth of self-detected breast cancers is also best described by a power law. The generality of the power law suggests that similar biological constrains (in particular angiogenesis and growth factors) operate in the growth of both self-detected and screened breast cancers and support a uniform approach to treating the disease. The implications of this growth law for both hormone and chemotherapy treatment will be discussed.

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480 Implantation and Treatment of a Chemoresistant Breast Cancer Cell Line in a Mouse Model.

Frühauf JH, Volz-Köster SR, Schmidt TJ, Förster C, Schneider J, Volz JO.

Introduction: Resistance to chemotherapy frequently poses a problem in the treatment of breast cancer. Chemoresistance is mostly associated with treatment failure, as its diagnosis is based on clinical detection of recurrent and/or progressive disease. The aim of the present study is to examine the molecular basis of chemoresistance in a strain of human breast cancer cell lines in an in-vivo model.

Methods: Native and chemoresistant cell lines of human breast cancer (native: mcf-7, resistant: mcf-7R-NOV, resistant to novantrone) were inoculated intraperitoneally into nude mice (n=160) In eighty animals, a CO2-pneumoperitoneum was applied over 30 min. after inoculation to enhance tumor cell adhesion to peritoneal epithelium. Five days after inoculation, therapeutic doses of novantrone were applied intraperitoneally. Animals were sacrificed two weeks after treatment and tumor tissues were snap frozen until further processing. Isolation and purification of mRNA from tumor samples were performed using the Trizol(R) and Dynabeads(R) methods. After transcription of mRNA (rtPCR) and radioactive labelling, differential gene expression analysis is performed using a cDNA array carrying 30.000 genes.

Results: Mice inoculated with mcf-7R-NOV and treated with CO2 pneumoperitoneum showed the greatest tumor burden at evaluation. Lowest tumor burden was found in animals inoculated with (native) mcf-7 not treated with pneumoperitoneum. Among the groups, mcf-7R-NOV showed lower tumor growth inhibition after therapy. In all groups, mice implanted with the mcf-7R-NOV showed reduced response after therapy indicating clinical chemoresistance. These differences in clinical response were also represented by differential gene expression patterns as evaluated by cDNA array examinations.

Conclusion: This animal model allows to verify chemoresistance of in-vitro prepared human breast cancer cells in vivo. An in-vitro resistant cell line showed significantly reduced response to cytotoxic treatment after intraperitoneal implantation. Using a cDNA array technology, the molecular differences of the resistant and native tumors can be demonstrated.

501 MRI Imaging Can Predict Breast Conservation in Patients Undergoing Neoadjuvant Chemotherapy.

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Introduction: Patients with locally advanced breast cancer who undergo neoadjuvant therapy may be able to avoid a mastectomy if their tumor shrinks during therapy. MR imaging patterns may help to predict which patients will be able to undergo breast conservation. We asked whether imaging patterns, hormone receptor status, and histology was predictive of response to therapy and ability to achieve a reduction sufficient to enable breast conservation.

Methods : 33 patients who underwent neoadjuvant chemotherapy for Stage 2 and 3 breast cancer had serial MRI imaging (before and after chemotherapy). Chemotherapy consisted of 4 cycles of Adriamycin and Cytoxan. Response to therapy was measured by change in longest diameter on MRI and physical exam. Patients were categorized according to four imaging types: dominant nodule with rim enhancement; infiltrating pattern (nodular or diffuse); patchy enhancement; and septal spread.

Results: After 4 cycles of AC chemotherapy, longest tumor diameter as measured by MRI strongly correlated with tumor size at the time of surgical resection (Spearman correlation coefficient=.84, $p<0.0001$; regression analysis $r2=0.92$, $p<0.0001$). 16 of 33 patients were able to undergo successful breast conservation after chemotherapy whereas only 2 of 33 were estimated to be able to have breast conservation prior to therapy. 75% of the patients able to undergo breast conservation were pattern 1. 88% (12/13) of the patients with pattern 1 were able to undergo breast conservation. Patients with patterns 2, 3, 4 had breast conservation rates of 28% (4/14), 0%(0/3) and 0% (0/4). 2 of the 14 patients in pattern 2 were assessed clinically as being able to have breast conservation prior to neoadjuvant chemotherapy. MRI patterns were associated with distinct histologic features. Pattern 1, 2, 3, and 4 were high grade invasive ductal(46% ER+), mixed ductal and invasive(79% ER+), lobular (100% ER+), and inflammatory (25% ER+), respectively.

Conclusion: Categorizing tumors by MRI patterns improved the ability predict the success of breast conservation by 85% ($p<0.0001$). High grade rim enhanced nodular tumors were most likely to respond and shrink sufficiently to enable treatment with lumpectomy.

502 Image Detected Lobular Neoplasia of Breast: Morphologic Correlation with Imaging Lesions.

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Lobular neoplasia (LN) is considered a lesion of risk for breast carcinoma that is classically an incidental finding on biopsy. However, lobular neoplasia has been reported as a primary lesion with image guided large gauge core needle biopsy (LGCNB). However, the incidence of LN as the cause of mammographic lesions has not been established.

2280 sequential image guided LGCNB were reviewed for a primary diagnosis of lobular neoplasia (LN). Cases were classified according to type of imaging abnormality [mass, calcification (Ca+), asymmetric densities (AD), shadowing (SH)]. Cases were reviewed and classified as to whether the LN was primary or incidental. If the LN was incidental, the type of primary lesion was also recorded.

One hundred eight LGCNB had a diagnosis of lobular neoplasia. 86 of these presented as calcifications (Ca+), 16 as masses, 5 as shadowing and 1 architectural distortion. Thirty-eight lesions of LN were primary (cause of mammographic lesion) and 70 were incidental. 32 primary LN cases were biopsied for Ca+ and 6 were masses. 54 incidental lesions of LN were biopsied for Ca+, 10 for masses, 5 for shadowing and 1 for AD. 32 primary LN were biopsies for Ca+ and 6 for masses. Incidental LN was associated with 17 cases of ductal hyperplasia, 15 duct carcinoma in-situ, 13 infiltrating lobular carcinoma, 9 radial scars, 6 mixed lobular-duct infiltrating carcinomas, 4 sclerosing adenosis, 3 infiltrating duct carcinomas, 1 papilloma, and 1 apocrine metaplasia. 8 of 10 mass lesions associated with incidental LN were malignant (6 infiltrating carcinomas and 2 DCIS).

Calcification was the most frequent cause for biopsy in 86/108 LN cases studied. Incidental LN was twice as frequent as a primary lesion of LN. The proportion of mass lesions was the same for both primary and incidental LN. However, mass lesions with incidental LN were frequently malignant (8/10). Almost one-half of lesions producing Ca+ associated with incidental LN were malignant (45%).

503 Mammographic Patterns and Breast Cancer Risk.

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Aim: To evaluate the risk of breast cancer with respect to mammographic density as described by Wolfe who classified the mammograms into N1, P1 codes (less density) and P2 and DY codes (increased density).

Patient and Methods: In 1980 a prospective study of symptomatic breast patients, attending the breast clinic, was set up to determine whether patients with Wolfe code P2 or DY had a higher risk of developing breast cancer than those with N1 or P1 codes. A total of 5021 women between 1980 and 1985 had their mammograms coded according to Wolfe classification. We present our interim results of the analysis of this cohort. From the pathology records of the hospital and crematorium records form district crematorium, we have matched the cases that had a diagnosis of breast cancer with our cohort to identify patients that developed interval breast cancer.

Results: We have identified a total of 280 patients in the initial cohort who have developed breast cancer. The distribution of these patients according to Wolfe coding was as follows: N1 = 42, P1 = 51, P2 = 143, DY = 44. The overall proportion of low risk codes (N1 and P1) was 33% and that of high risk codes (P2 and DY) was 67%. The proportion of these codes in the overall sample of 5001 evaluable patients was 51% and 49% respectively. This difference was statistically significant.

Conclusions: The interim results are in line with the earlier reports that high density Wolfe coding increased the risk of breast cancer.

504 Diffusion-Weighted MRI for Monitoring Treatment Response in Breast Tumors.

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Introduction: Magnetic resonance imaging (MRI) has proven to be valuable for the detection and treatment of breast cancer. Breast MRI is usually performed using contrast-enhanced techniques, which demonstrate tumor vascularity. Diffusion-weighted MRI (DWI) measures the apparent diffusion coefficient (ADC) of water in tissue. DWI relies on different physiologic mechanisms than contrast-enhanced methods, and may provide new and useful information about the tissue. The purpose of this study was to investigate whether treatment-induced changes in breast tumors were measurable using DWI.

Methods: 15 patients who underwent neoadjuvant chemotherapy for locally-advanced breast cancer had serial MRI exams (before, during, and/or after treatment) consisting of both contrast-enhanced and DWI acquisitions. Chemotherapy consisted of 4 cycles of adriamycin and cytoxan. ADC values were measured for tumor, uninvolved benign tissue (defined as non-contrast-enhancing parenchyma), and cysts in the patient scans.

Results: There were significant differences ($p<.05$) found between the mean ADC for tumor, benign tissue, and cysts (0.96 ± 0.21, 1.35 ± 0.29, and $2.19\pm0.37 \times 10^{-3}$ mm^2/s, respectively) in untreated patients. Preliminary results from the women undergoing neoadjuvant chemotherapy demonstrated a significant increase in ADC with treatment ($p=.02$). The average increase in tumor ADC was 12% after the first cycle of chemotherapy, and increased to 25% after 4 cycles. There was also a trend of decreasing ADC with treatment observed in the normal tissue measured in these patients, though this has not yet reached statistical significance.

Conclusions: The results of this study suggest that early tumor response to chemotherapy, in the form of cell death and necrosis, may be measurable in patients undergoing preoperative treatment using diffusion-weighted MRI. ADC values for tumor were significantly lower than for normal tissue, and a significant increase in tumor ADC was observable after only a single cycle of chemotherapy. In combination with current contrast-enhanced methods, DWI may improve the characterization of breast disease and could prove to be a more sensitive measurement of response to therapy.

505 Value of a Negative Scintimammography in the Evaluation of Patients with Abnormal Mammograms.

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We reviewed our experience with scintimammography involving more than 600 patients from 1997 to the present to assess the diagnostic ability of this procedure in detecting breast cancer. We identified 175 patients with mammograms suspicious or indeterminate for malignancy. These patients were selected irrespective of a palpable breast abnormality.

Scintimammography was performed using techetium 99m sestamibi, commercially known as Miraluma. The patients were injected in the dorsal vein of the foot. They were scanned in the prone position on a special mattress, which allows the breast to be suspended free. The scans were performed when the hormonal influence on the breast is the lowest (about the 10th day of the start of the menstrual period). Images were acquired with a gamma camera using a high-resolution collimator. Routinely, three images were obtained for a preset time. Additional images were obtained, if needed. The images were analyzed both digitally on a computer monitor and a radiographic film. All patients underwent stereotactic core biopsy or an open biopsy of the breast. We compared the biopsy results with the findings of scintimammography.

The results were as follows: True positive scans 57, true negative scans 107, false positive 5 and false negative 7. The sensitivity was 89%, specificity 96%, positive predictive value 92% and negative predictive value 94%. Based on our experience, we feel that negative nuclear scintimammography plays an important diagnostic role in the management of patients with suspicious mammograms.

506 The Role of Contrast-Enhanced 3D-MRI of the Breast in Detecting Local Recurrence after Surgery, Chemotherapy and Radiotherapy: Preliminary Results.

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Magnetic Resonance Image (MRI) is emerging as a novel and efficient method in breast diagnosis; the main indications are constituted by: evaluation of the patients with silicone implants (with or without mastectomy); follow up after tumorectomy; study of nodal involvement and post-surgical scars; early detection (presurgical and after radiotherapy) of plurifocal malignant lesions; clinical evaluation after neoadjuvant chemotherapy). Our study was performed on 100 patients, 99 females and 1 male. Sixty-eight patients with silicone implants were enrolled: 64 with mastectomy and 4 without; four pts were evaluated after breast conservation therapy, 2 for axillary nodes metastases from an unknown primary tumors, 20 for postoperative scarring and finally 6 pts were investigated to exclude multifocality. MRI imaging was carried out at least 6 months after radiotherapy. The MRI examination have been performed by a superconducting high-field magnet (Siemens 1.5 TESLA). The imaging sequences were: T1 turbo-IR scan time 6'30"; T2 fat suppression scan time 3'29", thickness of slice 3mm. After no contrast T1 and T2, a 3D sequence has been performed before and after contrast medium (gadolinium) with a matrix 205x205, scan time 1'11", minimum slice 2 mm. Visual evaluation of static enhancement was performed on subtraction images from precontrast and postcontrast series 2, 4 and 8 min after bolus injection. Any area of focal enhancement was manually examined (RO1) and had a time-signal intensity profile calculated. The curve was after evaluated according to the established criteria of contrast enhancement: early maximum signal and washout effect, with morphological criteria of malignancy, were considered strongly suspected (positive). The preliminary data show an excellent sensitivity and specificity for this innovative approach in the diagnosis and follow up of breast lesions: in all the 6 cases positive with MRI technique the biopsy confirmed the local recurrence suggesting the possibility to use routinally MRI to detect local relapse of breast cancer; no other technique including angio-power ultrasonography was conclused in this six true positive cases. But it seems that this novel clinical approach could be the gold standard to better define the therapeutic decision in primary breast cancer after neoadjuvant chemotherapy.

507 Mammographic Appearance of Nonpalpable Breast Cancer Predicts Pathologic Characteristics.

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The clinical behavior of breast cancer is due to its pathologic characteristics. Since the pathologic characteristics of breast cancer may be reflected by the mammographic appearance, we correlated the mammographic appearance and pathologic characteristics of 543 nonpalpable malignancies diagnosed in a single institution (MSMC) between July 1993 and July 1999. Cancers were divided into four groups based on mammographic appearance: a.) mass, b.) calcification, c.) mass with calcification (M&C) and d.) architectural distortion (AD). The majority of masses (95%), M&C (68%) and AD (79%) were due to invasive cancers, whereas the majority of calcifications (68%) were due to ductal carcinoma in situ (DCIS) ($p<0.001$). Among invasive cancers, calcifications were associated with necrosis (73%, $p<0.001$), extensive intraductal carcinoma (54%, $p<0.001$), and immuno reactive Her2/neu (44%, $p=0.012$). Calcifications on mammogram were associated with necrosis in DCIS (94%, $p<0.001$). Sixty-nine percent of DCIS associated with invasive cancers presenting as calcifications were EORTC high grade ($p<0.001$). Lymphatic invasion was common (46%) in cancers presenting as a M&C ($p<0.001$). Two-thirds of patients with AD had positive margins (65%, $p<0.001$) compared to 35% of cancers with other mammographic appearance. The ratio of invasive to noninvasive malignancies increased progressively with increasing age ($p<0.001$) from 51% at <50 years to 76% at 70-80 years, while the ratio of masses to calcifications changed from 30% at <50 years to 70% at 70-80 years ($p<0.001$). Mammographic calcifications associated with malignancies are due to DCIS alone or DCIS accompanying the invasive component, while mammographic masses are almost always associated with invasive cancers. When invasive malignancies presented as calcifications, the calcifications were associated with accompanying high grade DCIS with necrosis, and they were often Her2/Neu positive. These results indicate that the mammographic pattern of malignancies can be used to predict pathologic characteristics of the cancer.

508 Radionuclide Imaging of Human Breast Carcinoma Cell Line MDA-MB-231 Via In-111 DTPA-Adenosylcobalamin, In-111 DTPA-Octreotide, Ga-67 Citrate, and Tl-201 Chloride.

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The purpose of this study was to visually and quantitatively compare the *in vivo* biological activity of the vitamin B12 analog In-111 DTPA-Adenosylcobalamin (In-111 DAC) to In-111 DTPA-Octreotide (In-111 OCT), Ga-67 citrate, and Tl-201 chloride in human breast carcinoma cell line MDA-MB-231 transplanted in nude mice.

To accomplish this, MDA-MB-231 was grown in tissue culture, harvested, and inoculated into the left flank of nude mice. Once tumor size reached one centimeter, approximately 100 µCi of either In-111 DAC, In-111 OCT, Ga-67, or Tl-201 was administered intraperitoneally. A minimum of three mice per radiopharmaceutical were studied. At 24 hours after injection, post-mortem planar images were acquired for 20 minutes. The activity of dissected organs and tissue was measured by gamma well counter. Counts per minute per milligram of tissue (cpm/mg) were calculated and compared among the four radiopharmaceuticals. Mean (range) cpm/mg for each of the four agents are as follows:

In-111 DAC	In-111 OCT	Ga-67	Tl-201
5342 (1129)	55 (24)	2573 (20)	2046 (226)

Both visually and quantitatively, In-111 DAC was superior to the other agents in imaging human breast carcinoma cell line MDA-MB-231. This was also true in imaging human cell lines of bronchogenic (A549), colon (LS 174T), and pancreatic carcinoma (Capan-1), as well as rhabdomyosarcoma (A673) transplanted into nude mice (data not shown).

In-111 DAC may prove to be a useful tumor imaging agent. Further work to evaluate the clinical potential of In-111 DAC in scintimammography is warranted.

509 Diagnostic Value of Mammography and Breast Ultrasound for Diagnosis of Non-Palpable Lesions of the Breast.

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In these study, we attempted to demonstrate the usefulness of breast ultrasound in the detection of non - palpable breast lesions.

We studied 360 patients (mean age 54,7 years) undergoing excision of non - palpable lesion detected by mammography or breast ultrasound between december 1997 and december 1999. The imaging diagnoses were compared with the histology results using four - field contingency table. The success of mammographic and sonographic localization was analyzed retrospectively.

The 360 patients had 64 invasive carcinomas, 102 ductal carcinomata in situ (DCIS), 2 lobular carcinomata in situ (CLIS) and 192 benign lesions. 50 (78,1%) of the invasive carcinomas were initially found with mammography, 14 (21,9%) with sonography. The sensitivity and specificity for malignancy were 85% and 34,8% for mammography, 92,2% and 38,2% for sonography and 98,4% and 12,2% for combination of both methods, respectively. 10 of the invasive carcinomas were labelled via radiography and 54 sonographically (complete removal in all cases).

Mammography is the prefered screening method. Breast ultrasound can identify lesions in almost all breasts with mammographically detected invasive carcinomas. High - resolution techniques (3 D, contrast agents - Levovist) are used to determine non - palpable invasive carcinomas and preinvasive lesions. The combination of ultrasound and mammography improves diagnostic sensitivity, particular in premenopausal women with radiodense breast. Ultrasound - guided localization simplifies intraoperative removal of lesions in comparison with radiographic localization.

510 FDG-PET in Preoperative Assessment of Newly Diagnosed Breast Cancer.

Schirrmeister HH, Kuehn T, Buck AC, Santjohanser C, Reske SN.

The aim of this study was to examine if one FDG-PET scan provides the complete information needed prior to surgery. The accuracy of FDG-PET was compared with the accuracy of the standard staging methods (physical examination, mammography, ultrasonography, bone scans and radiography of the lung). Methods: 117 female patients were included into this prospective study. Patients were selected when suspicious breast lesions were present at palpation, ultrasonography or/and mammography. A high resolution full-ring scanner (Siemens ECAT HR+) was used for PET imaging. Patients (fasted for > 8 hours) were placed in a prone position with elevated arms 1 hour after injection of 370 MBq FDG. The PET scans covered the neck, breasts, thorax and liver in all patients. Results: The sensitivity in detecting axillary nodes with FDG-PET was 79% (specificity: 92%) compared to a sensitivity of only 41% with palpation (specitifity 96%). FDG-PET indicated internal lymph node metastases in 12 patients and a metastasis at the contralateral axilla in one patient which were not revealed by the standard staging methods. Sensitivity (63%, specificity: 96%) was also two-fold in detecting multifokal cancer using FDG-PET compared with the combination of palpation/ ultrasonography/ mammography (sensitivity: 32%, specificity: 93%). In addition, FDG-PET was true positive for lung metastases in 4 patients. By contrast planar x-ray was negative in 3 of these patients. Conclusions: Histological evaluation of the axillary lymph node status cannot be replaced by FDG-PET due to a false negative rate of 20%. But there might be an improvement of patients management by the detection of internal nodes, multifokal cancer or lung metastases missed by the standard staging modalities.

511 Delay to Diagnosis Worsens Prognosis of Screen-Detected Breast Cancer.

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Delay to diagnosis of a breast screening abnormality causes anxiety but it is unknown if it also worsens prognosis. Using pooled data from 5 Canadian organized breast screening programs, we evaluated prognosis in 4465 invasive breast cancers diagnosed (dx) in the ipsilateral breast within 3 years of an abnormal screen performed during 1990-1996.

Women with 'high suspicion' screens (N=1579) compared to 'other' cases were more promptly investigated (median time from screen to dx = 31 and 47 days; p=0.0001), had larger tumors (21% vs 44% ≤10mm; p=0.0001) and were more likely to be node-positive [N+] (33% and 16% N+; p=0.0001). The table shows the distribution of 'other' cases and the proportion which were N+, ≤1cm or >2cm during various times to diagnosis after an abnormal screen.

Interval	≤ 4 wks	5-12 wks	13-20 wks	21-52 wks	1-2 yrs	2-3 yrs
Number	539	1618	172	189	119	49
Node +	18%	15%	16%	19%	32%	43%
T≤10mm	37%	47%	49%	46%	35%	22%
T>20mm	17%	14%	10%	16%	26%	20%

Delays to diagnosis beyond 12-20 weeks are associated with increasing tumor size and node positivity. Physicians expedited diagnosis for those with high suspicion, worse prognosis screens. This 'suspicion bias' obscures whether delays <20 weeks also worsen prognosis and should be taken into consideration in analyses of the effect of delay on prognosis.

512 Screening Mammography Program of British Columbia (SMPBC): 10 - Year Outcomes.

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The SMPBC is a population-based, organized breast-screening program in BC, Canada that has provided bilateral, 2-view mammograms, every 1 to 2 years, free of personal charge to asymptomatic women age 40 and older since 1988. Women younger than 40 and older than age 80 may attend with a physician's referral. Women age 40-79 may book their own appointments.

10-year screening outcomes were evaluated using prospectively collected data for screens provided between 1988-1997 inclusive. Rates of participation, abnormal referral, cancer detection and interval cancer were evaluated. A cancer was a ductal carcinoma in situ or any histological type of invasive breast carcinoma.

There were 895,849 screening mammograms provided to 335,433 women. 51.3% of women were age 50-69 years. Abnormalities were identified on 57,454 screens (6.4%) from which 3304 cancers were detected. Abnormal call rates were higher on first (9.8%) as compared to subsequent screens (4.4%) and declined with age: 7.7% at age <40 to 5.4% for age 70-79 years. Cancer detection rates were higher on first (5.0 per 1000) as compared to subsequent screens (2.8 per 1000) and increased smoothly with age from 1.4 to 8.2 per 1000 from age <40 to age 80 years and older. Twenty percent of cancers were non-invasive. The median size of invasive cancers was 14 mm and 81% had no axillary lymph node metastases. The 12-month interval cancer rate was 0.6 per 1000 and did not vary significantly with age or screening history.

Across a broad range of ages including large numbers of women outside traditional target ages for organized breast screening programs, surrogate indices of screening mammography success have been achieved in a population-based, North American, organized breast screening program.

513 The Role of Risk Factors on Multi-State Natural History of Breast Cancer: Implications for Breast Cancer Screening for Female Relatives of Breast Cancer Index Cases.

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In 1998, we published a paper demonstrating the efficacy of breast cancer screening for high-risk group using data from Taiwan Multicenter Cancer Screening (TAMCAS) (Int J Cancer 1998; 78: 21-26). Recently, we are interested in whether the efficacy of breast cancer screening for this high-risk group, female relatives of breast cancer index cases, depends on different established risk factors including age at menarche, menopause, age at first full-term pregnancy (AFFTP), obesity and smoking. The above risk factors may act either as initiators for onset of preclinical breast cancer or as promoters for triggering the progression from the preclinical screen-detectable phase (PCDP) to clinical phase or both. The aims of this study are to examine the effects of relevant covariates on preclinical incidence rate and transition rate from the PCDP to clinical phase. The above estimates are applied to predict the efficacy of screening on mortality reduction given a combination of significant factors. The data used in this study are from a high-risk group screening project, TAMCAS. Details of original study design have been published elsewhere (Int J Cancer 1998; 78: 21-26).

Results show that significant factors accounting for onset of preclinical breast cancer include smoking, AFFTP, and obesity. Relative risks for smoker versus non-smoker, late AFFTP, versus early AFTTP and the obese versus the non-obese are estimated as 5.00 (1.20-20.88), 3.52 (1.46-8.51), and 3.75 (2.06-6.86), respectively. As regards promoters, AFTTP and obesity play important roles in the progression from the PCDP to clinical phase. Average duration of the PCDP for late AFFTP is approximately 1.32 years as compared with 4.89 years for early AFFTP. The corresponding estimate for the obese (1.54 year) is only half that for the non-obese (3.31 year). The application of these estimates to evaluating the efficacy of annual screening for four combinations of AFFTP and obesity yields the predicted mortality reduction: 12%, 39%, 40%, and 46% for the non-obese with early AFFTP, the non-obese with late AFFTP, the obese with early AFFTP and the obese with late AFFTP, respectively. Results suggest screening frequency of breast cancer screening for female relatives of breast cancer index cases may take obesity and AFFTP into account.

514 Breast Health Access for Women with Disabilities: Challenges in Screening Women with Physical Disabilities.

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In order to increase awareness among health professionals this poster will offer a review of the development of a breast health screening program targeting women with physical disabilities who are age 20 and older.

In December 1994 the Breast Center became aware that women with disabilities experience attitudinal, educational and logistical barriers that limit their access to breast screening. Searches failed to identify any programs in existence with an emphasis on screening for women in wheelchairs or those with serious motor, muscular and neurological conditions. In 1995 a working conference was held to bring together the leaders of the local disability and medical community. This provided extensive information and led to a needs assessment that helped define the program. A clinic was opened in 1997 with a MammaCare® trained nurse, to provide clinical breast examination (CBE) and breast self examination (BSE) instruction for women with disabilities. An exam table was purchased that lowers to 19" off the floor and allows many women to transfer without assistance. The purchase of a special mammography unit with tilting capability and designed without a pedestal allows imaging of most women in their wheelchairs. Through questionnaires filled out by the client prior to the first visit we learned that most women with disabilities have not had a CBE as part of their health care. Screening mammography has not been recommended for this population. Outreach to this population is more complex because these women do not share a common language or ethnic background. The goal of the clinic is to screen and educate women and to document their ability to participate in doing BSE. We are in year 2 of a 3 year BCRP research grant to collect and analyze data from 1,000 women with physical disabilities who live in Alameda or Contra Costa counties. A manual is being developed to assist other sites in establishing programs to improve access.

515 BRCA1/2 Genetic Testing in Spain: A Search for Recurrent Mutations.

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Frequent founder mutations of BRCA1 or BRCA2 have not been reported in Spanish breast cancer (BC) and ovarian cancer (OC) families, possibly reflecting the ethnic heterogeneity of the Spanish population. However, a large comprehensive survey of high-risk BC/OC families from this population has not been done, and it remains possible that a limited number of mutations account for most hereditary cases. The aim of this study is to determine the prevalence and distribution of BRCA1 and BRCA2 mutations in BC/OC families from different ethnic groups in Spain.

Diagnostic criteria for “high risk” families included at least 2 members with early onset BC or one member with BC and one with OC. Since November 1998 we visited 215 famlies with a family history of breast cancer, and 118 of them fit the high risk criteria. So far, the entire coding regions of BRCA1 and BRCA2 have been directly sequenced in 20 families. When no mutation was found, Southern blot methodology was used to detect large deletions or rearrangements. Ten families had deleterious mutations including one large rearrangement in BRCA1, 5 truncating mutations in BRCA1, and 3 truncating mutations in BRCA2. In addition one family had a BRCA2 missense variant that is not a polymorphism in the Spanish population and was previously reported in the Breast Cancer Information Core as a deleterious mutation. Two of the BRCA1 mutations, 189insTGTC and 5537delA, were previously reported in Spanish families. The 10 mutations found in this study comprise 1/3 of all mutations reported in Spain. The frequency of positive results (10/20) in our study with complete sequencing of these genes is higher than previously described in Spanish population selected with similar criteria and analyzed by Single Strand Conformation Polymorphism analysis (SSCP) and Protein Truncation Test (PTT). That two out of 10 mutations were recurrent supports the further analysis of this population for moderately common founder mutations and the correlation of specific mutations with ethnic subgroups.

516 Predictors of Genetic Counseling for BRCA1/2 Among Unaffected Women

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Physicians may be faced with requests for BRCA1/2 testing from women without a prior cancer diagnosis. There are multiple reports of decision making, intent to test, and the impact of BRCA1/2 counseling and testing among women with prior cancer diagnoses. Unaffected women have not been similarly studied, yet this is a group likely to seek testing and who could potentially benefit greatly from genetic counseling. We mailed a questionnaire followed by printed BRCA1/2 education materials and a follow-up survey to 87 women identified by clinical referral who met these eligibility criteria: estimated 10% probability of carrying a BRCA1/2 mutation, no previous cancer genetic counseling, and no tested relatives. The 42 women (48% response rate) completing both questionnaires were offered free genetic counseling. We compared those who accepted the counseling to those who did not. Respondents were mostly Caucasian (98%), college educated (74%), and insured (95%); 95% had ≥ 1 first-degree relative with breast or ovarian cancer. Using standard scales, neither depression nor anxiety were elevated in the group as a whole. On a five point scale (1=strongly agree, 5 strongly disagree) participants’ perceptions of testing benefits (mean= 3.8, s.d.=0.6) were stronger than their perceived barriers (mean=2.3, s.d.=0.4). In general, the group was adherent with mammographic screening, with 88% planning mammography on at least a yearly basis or when recommended by their doctor. We found no differences in these parameters between those choosing/declining counseling. We did find differences in baseline and post-educational material knowledge between those choosing/declining counseling. On a 13-item true/false knowledge scale, those choosing counseling scored better than those declining both at baseline (54% vs.38%, p=0.014) and after materials (85% vs. 54%,p=0.018). We also found differences in prophylactic options considered: those choosing counseling were more likely to have had or considered an oophorectomy (50% vs. 13%, p=0.02) and tended toward being more likely to have had or considered mastectomy (54% vs. 31%, p=n.s.). These data suggest that unaffected women with more knowledge who are considering prophylactic interventions are more likely to accept genetic counseling.

517 Histologic Abnormalities in BRCA 1 and BRCA 2 Mutation Carriers Undergoing Prophylactic Mastectomies.

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Women with BRCA1 and BRCA 2 mutations have a cumulative risk of breast cancer ranging up to 85 percent. Early reports have suggested that women with BRCA related breast cancers show less ductal carcinoma in situ around the invasive lesions than do control patients with breast cancer. In order to study the evolution of breast cancer in BRCA mutation carriers, we performed a retrospective review of the Breast Surgery Database at the New York Presbyterian Hospital-Presbyterian Center between 1998-2000 in order to identify women with BRCA mutations who underwent prophylactic mastectomies. A total of 8 prophylactic mastectomies were performed in 5 patients. All patients were asymptomatic with normal physical exams at the time of surgery and had undergone routine breast imaging studies which were without evidence of malignancy. Age ranged from 31-51 years with a mean of 39.8 years. Three patients had a previous personal history of breast cancer more than five years prior to undergoing prophylactic surgery. Two of these patients had been treated with modified radical mastectomies and one had undergone breast conservation. Pathology from five of the eight specimens revealed unremarkable fibrocystic changes. One patient without prior history of breast cancer showed atypical ductal hyperplasia in the right breast and proliferative fibrocystic changes in the left breast. Finally, one patient with a history of contralateral breast cancer had a single focus of ductal carcinoma in situ as well as several foci of lobular neoplasia. In summary, even in this small series, significant histologic abnormalities were identified in BRCA mutation patients undergoing prophylactic mastectomies, ranging from atypical ductal hyperplasia to ductal carcinoma in situ. Contrary to previous reports, we conclude that BRCA mutation carriers may not have a different progression from hyperplasia to in situ carcinoma to invasive breast carcinoma when compared with non-mutation carriers.

518 Descriptive Study on the Use of Prophylactic Surgery in Women with Known BRCA Mutations: The Mount Sinai Hospital Experience.

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Rationale: Women with BRCA1 or BRCA2 have an increased lifetime risk for both breast cancer (BC) (up to 85%) and ovarian cancer (OC) (up to 60%). At present, women with BRCA mutations in either gene can be managed by intensive screening (with or without chemoprevention) or by prophylactic mastectomy (PM) and/or prophylactic oophorectomy (PO). Our primary study objective was to identify factors important in decision-making regarding risk management. We also evaluated patient's satisfaction with their decision and the counseling process (pre test/post result), their risk perception and coping abilities. **Methods:** A semistructured interview was conducted (in clinic or by telephone). Three psychological questionnaires were also administered. **Results:** A total of 23 women with known BRCA mutations were evaluated: median age, 53 years (range:35-79 years); previous BC in 19 women with no known systemic recurrence; median interval from receipt of test result to interview: 488 days (range 61-1308 days). Eleven women had PM (9 had previous BC). Of those, one had PM years prior to genetic testing. Twelve women opted for intensive screening (10 had previous BC). In those women who did not have previous bilateral mastectomies (therapeutic or prophylactic), the median perceived risk of future BC was 40% (range:0-100%) whereas women who had previous PM perceived their median risk of future BC to be 30% (range: 0-80%). PO was performed in 14 patients of whom 3 were simultaneously investigated for benign conditions. Six additional women are planning to have PO. Intense fear of cancer rather than lack of confidence in the screening program was the primary motivating factor to undergo PM or PO in most women. Of the women who had PM or PO, none had any regrets although some reported reduced energy level initially or emotional distress. All patients said they would undergo genetic testing again if they were to start over. Details of patient satisfaction, risk perception and coping abilities will be presented at the meeting. **Conclusion:** Women with BRCA mutations often opt to have prophylactic surgery based on an intense fear of cancer. They appear satisfied with their decision.

519 Graphical Risk Explanation as a New Method of Explaining Risk of Developing Breast Cancer to Women with a Family History - Randomised Controlled Trial to Evaluate Its Effect on Reducing Anxiety.

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Background: It has always been a matter of debate as to which is the best way of explaining risk to women with a family history of cancer. A woman's accurate understanding of her risk is important to alleviate risk-related anxiety. However, it is not known whether risk has to be explained in a non-numerical or numerical format and if numerical whether it needs to be in the form of an odds ratio, relative risk or absolute risk. The most accurate method and also the most difficult to understand is absolute risk as it varies with age and different causes of mortality.

We have devised a graphical risk explanation method, which depicts absolute risk for differing family histories along with population risk at each age group. Absolute risk figures are shown for remaining lifetime and for the next ten years.

Aim: To compare the effects of graphical risk explanation and verbal risk explanation on objective anxiety scores 6 weeks after counselling for familial risk of breast cancer.

Methods: 50 women who received counselling at the EGA Hospital, were randomised to receive graphical risk explanation or verbal risk explanation. Spielberger state-and-trait anxiety assessment questionnaires were administered to all women immediately before the counselling session and mailed to them 6 weeks after the counselling session. Difference in anxiety scores pre-and-post counselling were tested for significance in each group using Wilcoxon's signed rank test.

Results: 21/25 of those having graphical risk explanation and 13/25 of those having verbal risk explanation returned their post-counselling questionnaires. There was a significant fall in state anxiety scores in those receiving a graphical risk explanation ($p=0.03$) and no difference in anxiety in those receiving verbal risk explanation.

Conclusion: A graphical explanation of risk can reduce risk-related anxiety levels by enhancing visual understanding of risk.

520 Breast Density (BD): Association with Risk Factors and Stage at Diagnosis.

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BD is thought to impair mammographic screening, and has been proposed as a surrogate biomarker for risk. We sought to examine the relationship between BD, stage at diagnosis, and known risk factors in 167 patients with screen detected cancer (106 invasive, 61 DCIS). Mean patient age was 58 years (range 38-91), and 51% of the lesions contained calcification. Density was classified by BIRADS categories of fatty (n=40), mixed (n=85), or dense (n=42) by a single observer. Analysis was by Fisher's exact test or logistic regression, and results are expressed as odds ratio (OR) with 95% confidence intervals (CI). 60% of cancers occurring in dense breasts were DCIS, but DCIS accounted for only 23% of tumors in fatty breasts. Overall, DCIS was 3X more likely to occur in dense or mixed breast tissue than in fatty breasts (OR 3.15, CI 1.68-5.88, $p<0.001$). The relationships between density and risk factors are shown.

Variable	OR	95% CI	P-value
Age	0.92	0.89-0.95	<0.001
Age at first birth	1.10	1.04-1.16	<0.001
Any family hx	0.69	0.38-1.25	NS
Hx of 1° relative	0.51	0.25-1.02	NS
Prior breast bx	2.11	1.05-4.25	NS

We conclude that breast density does not impair the detection of DCIS, and should not be used as a rationale for avoiding screening. Increased density correlates with late age at first birth, but not family history or prior biopsies. Further investigation to see if density is a surrogate for the increased risk associated with long hormonal exposure is warranted.

521 Alteration of Stromal Protein Expression in Radial Scars of the Breast Assessed by mRNA In Situ Hybridization (ISH).

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We recently reported that radial scars (RS) are an independent histologic risk factor for breast cancer (N Engl J Med 1999;340:430). Although the reason for this association is not known, the formation of RS may be a reflection of a disturbance in the interaction between stromal and epithelial cells that is thought to be important in the pathogenesis of mammary carcinoma.

To address this issue, we performed in situ hybridization (ISH) on formalin-fixed, paraffin sections of 12 RS using ^{35}S-labeled riboprobes to determine the level of mRNA expression for several proteins whose expression is commonly up-regulated in invasive breast cancers, including collagen type 1, total fibronectin, ED-A+ fibronectin (a spliced variant strongly expressed in embryonic development) and thrombospondin-1. For each of these proteins, the level of expression of mRNA in the RS was compared with that of normal breast tissue on the same slide and with that of 4 invasive breast cancers.

Compared to normal breast tissue, the stromal cells in RS showed markedly increased expression of mRNA for collagen type 1, total fibronectin, ED-A+ fibronectin and thrombospondin-1. The level of mRNA overexpression for these proteins in the RS was similar to that seen in the 4 invasive cancers studied.

We conclude that there are striking similarities between RS and invasive breast cancers with regard to the level of mRNA overexpression for several stromal proteins. These results raise the possibility that a similar disturbance in stromal-epithelial interactions underlies the pathogenesis of both lesions. This may explain, at least in part, why RS are associated with an increased risk of breast cancer.

522 Breast Cancer Mortality and Pesticide Exposure in Brazil.

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Breast cancer is the first cause of cancer among women observed in several Brazilian cancer registries. This rise has been observed in the last two decades, and possible involved reasons have been searched. Pesticides exposure has been mentioned as associated to several cancer sites, mainly leukemia, brain cancer, lymphoma and soft tissue tumors. Hypothesis involving their association with breast cancer was also highlighted considering that some pesticides could act as endocrine disrupters enhancing estrogenic activity.

We carried out an epidemiological ecologic study evaluating population exposure to pesticides in eleven Brazilian states in 1985 and further breast cancer mortality (1997-98). The former was ascertained by pesticides sells (tons) reported by government registries in different geographic regions, and respective breast cancer mortality was obtained from national mortality data. Correlation analysis was carried out and correlation coefficients and their 95% confidence intervals determined for the general population, women 20-39 yr, 40-49 yr and 50-69 yr old. For all women, we observed a high correlation between past population exposure to pesticides and breast cancer mortality (r=0.74, 95% CI 0.27-0.93). Among women 50-69 yr, correlation coefficient was the highest observed (r=0.81, 95% CI 0.41-0.95).Among women 40-49 yr. and 20-39 yr. they were, respectively, 0.66 (95% CI 0.10-0.90) and 0.39 (0.0-0.80). Correlations involving exposure to specific chemical agents and breast cancer mortality will also be presented.

These results are in agreement with a possible association between pesticides exposure and breast cancer mortality (still high following breast cancer incidence), mainly after menopause. If true, breast cancer incidence will possibly maintain its rise in Brazil, since pesticides consumption has increased several folds since 1985. Nevertheless, ecological fallacy cannot be dismissed considering that individual exposure was not evaluated in this study. These observations encourage further research in Brazil aiming to evaluate past exposure to pesticides and female breast cancer as outcome.

523 Risk Factors for Breast Cancer Are Favorable Prognostic Factors.

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Women at high risk of developing breast cancer are commonly thought to have high risk of recurrent disease. The Gail model was used to divide 402 women with breast cancer into high risk and low risk groups. These patients were operated on and followed-up by the senior author (PIT) in a single institution between January 1981 and December 1995. The mean relative risk (RR) was 2.1.

Table 1. Significant differences between high risk and low risk breast cancer patients.

		High risk	Low risk	P
Number of patients		112	290	
Age at first birth (mean, years)		29	24	<0.001
Number of breast biopsies (mean)		28 (25%)	3 (1%)	<0.001
Family history of breast cancer		82 (73%)	0	<0.001
Race	White	91 (81%)	185 (65%)	0.014
	Black	8 (7%)	44 (15%)	
	Hispanic	12 (11%)	54 (19%)	
	Other	1 (1%)	3 (1%)	
Tamoxifen treatment		45 (43%)	158 (59%)	0.004
Distant disease-free survival				
	5-years	95.2%	86.6%	0.013
	10-years	91.6%	77.8%	0.006

There was no difference between the two groups in age, mean number of live births, age at menarche, presentation, mammographic results, fine-needle aspiration results, diagnostic method, histology, differentiation, tumor size, axillary nodal status, level of axillary nodal involvement, steroid receptor status, type of surgery (mastectomy or breast conservation), radiation therapy, chemotherapy, and local disease-free survival. Women at high risk for breast cancer have a better prognosis following diagnosis than women at low risk. This survival advantage is not due to differences in stage at presentation. Our data refutes the notion that risk factors for breast cancer are prognostic.

524 Atypia on Breast Fine Needle Aspiration (FNA): Implications for Breast Cancer Risk Assessment.

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Breast cancer risk assessment using the Gail Model (GM) is now widely used in clinical practice. The significance of atypia found on FNA in this setting is uncertain, but may impact the eligibility of women for clinical trials and prophylactic intervention. We have reviewed all FNA samples signed out as atypical at our institution over the past six years. The review was blinded to the original FNA diagnosis; 20 cases were added for purposes of blinding (10 previously diagnosed as benign without atypia, and 10 originally read as suspicious or malignant). The review was performed by a single cytopathologist, using well-defined cytologic criteria. All patients underwent subsequent surgical excision (SE) of the lesion. The original FNA (oFNA) diagnosis was compared to the revised FNA (rFNA) diagnosis, and the SE diagnosis. The cases that were added for blinding purposes were excluded from the final analysis. The impact of the oFNA, rFNA and SE diagnoses on the calculated 5 year breast cancer GM risk was determined.

The mean age of the 43 study subjects was 44 years. All patients had cytologic atypia diagnosed originally, reduced to 15 (35%) on revised diagnosis. Four women (9.3%) had atypical hyperplasia on SE.

5 year GM Risk	**oFNA**	**rFNA**	**SE**
median	2.15	1.5	1.3
(95% CI)	(0.3-6.2)	(0.5-4.8)	(0.3-4.0)
Risk > 1.66	27/43	19/43	15/43
(percent)	62.8	44.2	34.8

rFNA atypia diagnosed by a single cytopathologist using well defined criteria correlated better with SE than the oFNA diagnosis made by a variety of pathologists. There were significant differences in the 5 year GM risk between the groups ($p<.00001$ for the oFNA vs. SE, and $p<.01$ for the rFNA vs SE).

These data show that unless strict criteria are used by an experienced cytopathologist to diagnose atypia on breast FNA, the correlation with surgical pathology findings can be quite poor, and can inflate the GM estimates significantly. The long-term significance of atypia on FNA is not known. Therefore the use of FNA derived information for risk estimation requires caution, and needs validation before it is used widely in clinical management and in studies of prophylactic interventions.

525 Breast Cancer Risk Assessment: Correlation of Mammographic Patterns with Clinical Evaluation.

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Purpose: To evaluate the potential clinical utility of digital mammography and computerized assessment of mammographic patterns in the evaluation of women at risk for breast cancer. Materials and Methods: Mammograms from 503 women (380 with no cancer, 30 with BRCA1/BRCA2 gene mutation and 93 with breast cancer) were digitized at a pixel size of 0.1 mm and 10-bit quantization. Fourteen computer-extracted features were calculated from the central region of breast image to characterize percent dense of the breast or the heterogeneity (diffuse) patterns in the dense portions of the breast. Different approaches were employed to identify mammographic patterns associated with breast cancer risk as determined using the clinical risk assessment models (e.g., the Gail model and the Claus Model) and information on BRCA mutation status and cancer status. Selected features were merged to 1) correlate with risk as determined from the Gail and the Claus models using linear regression models, 2) correlate with risk from using a logistic regression model, and 3) differentiate between the BRCA mutation carriers and low-risk women using a linear discriminant analysis model. Results: The linear regression models achieved correlation coefficients (r) of 0.57 and 0.61 in predicting breast cancer risk as determined from the Gail and the Claus models, respectively. The linear logistic regression model yielded a correlation coefficient of 0.82 in predicting the observed breast cancer risk. The linear discriminant analysis model yielded Az of 0.89 in differentiating between BRCA1/BRCA2 mutation carriers and low-risk women. Conclusion: Computerized models using information from digital mammography may potentially aid in the assessment of breast cancer risk.

526 A Phase II Trial of Pemetrexed Disodium (ALIMTA™, LY231514, MTA) in Metastatic Breast Cancer (MBC) Patients Who Have Failed Anthracyclines (A) and Taxanes (T) (Salvage Chemotherapy).

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Pemetrexed disodium (ALIMTA™) is a potent inhibitor of multiple folate-dependent enzymes. Spielmann et al. (San Antonio 1999) noted a 21% ORR in 72 patients (pts) resistant/refractory to A treated with 600 mg/m2 pemetrexed every 21 days; 43% had also received T. We now report on 53 MBC pts treated with pemetrexed 500 mg/m2 IV, plus dexamethasone to prevent skin rash. Multivitamin therapy (MVT) was added after half of the patients were enrolled. All pts had received A and T, 28% pts were refractory to A (progressing within A therapy), 72% pts resistant to A; 45% pts were refractory and 55% were resistant to T. The pts best response to the last chemotherapy was CR+ PR (12%), SD (30%), and PD (58%). Median age was 56 years (range 31 - 72), median number of metastatic sites was 2 (range 1 - 6), and prior number of chemotherapies was 2 (range 1 - 4). Fifty percent of pts had liver metastases, and 29% had lung metastases. Pemetrexed was well tolerated with 33% and 15% of pts experiencing CTC grade (GR) 3/4 neutropenia but no febrile neutropenia, and no GR 3/4 thrombocytopenia. However, 2% pts had GR 3 infection. Clinical toxicity was mild, with 8% of patients experiencing GR 3 rash, and 2% experiencing GR 3 vomiting or fatigue. No pt had hand/foot syndrome. Two pts discontinued due to adverse events, not related to study drug.

As of Feb 2000, 32 pts are evaluable for efficacy, with 6 PRs (19%) and 17 SDs (53%). Full efficacy and safety results (53 pts) with and without MVT will be available at the time of the 2000 San Antonio conference. Pemetrexed is showing promising activity as salvage therapy in MBC after failure of A/T. A phase III trial is planned.

527 Trastuzumab (Herceptin) Combined with Weekly Paclitaxel in the Treatment of Metastatic Breast Cancer: A Phase II Study.

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Objectives: Taxanes are the most active drugs in the treatment of metastatic breast cancer. The weekly therapy with paclitaxel combined a high efficacy with a remarkably reduced toxicity.

The HER2/neu proto-oncogene is overexpressed in 25% to 30% of patients with breast cancer. Trastuzumab (Herceptin) a recombinant humanized monoclonal antibody with high affinity for the HER2 protein inhibits the growth of breast cancer cells overexpressing HER2.

In this phase II study the efficacy and toxicity of weekly administration of trastuzumab combined with weekly therapy of paclitaxel was evaluated in 24 patients with metastatic breast cancer whose tumor or metastases overexpressed HER2. Patients in this trial had extensive metastatic disease and most had received prior anticancer therapy.

Paclitaxel was administered as an one hour infusion in a dose of 90 mg/m² for twelve weeks. Trastuzumab was administered at a starting dose of 4 mg/kg followed by 2 mg/kg/week. Premedication consisted of dexamethasone 4 mg iv, clemastine 2 mg iv and ranitidine 50 mg iv 30 minutes before paclitaxel. All patients were evaluable for toxicity and response. The median age was 53 years. No severe hematological and non-hematological toxicities occured. No case of febrile neutropenia was observed. An alopecia grade III WHO developed in 10 of 24 patients. Some of them started with an alopecia resulting from earlier treatment. Among the 24 evaluable patients, twelve had tumor responses: five patients had a complete remission and seven had a partial remission. Therefore, the overall response rate (complete plus partial remission) was 50%. Nine patients had stable disease and we observed three patients with progress of disease. All patients with remission are still under treatment with weekly administration of trastuzumab until progression.

Conclusion: In this group of heavily pretreated patients we observed a remarkable activity of this treatment with a low incidence of toxic side effects. Hematological and non-hematological toxicities were rare. This weekly schedule with paclitaxel and trastuzumab is possible in an outpatient setting.

528 1α-Hydroxyvitamin D_5 Enhances Doxorubicin-Induced DNA Breakage in ZR75-1 Breast Cancer Cells.

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We have previously shown that 1α-hydroxyvitamin D_5 ($1\alpha(OH)D_5$), a non-calcemic analogue of Vitamin D_5, inhibits the growth of both ER-positive and ER-negative breast cancer cell lines in vitro and in athymic mice. Doxorubicin (Dox) is commonly used in the treatment of breast cancer. Its mode of action includes the generation of reactive oxygen species (ROS) and inhibition of topoisomerase (topo) II. Both these mechanisms cause DNA breakage. The current study was undertaken to evaluate the effect of pre-treatment of ZR75-1 breast cancer cells with vitamin D analogues, $1\alpha(OH)D_5$ (100 nM) and $1\alpha(OH)_2D_3$ (10 nM) on Dox-induced DNA breakage. The comet assay was used to evaluate DNA breakage 1 hr after treatment with Dox (0.1 μM, 0.3 μM, 1μM, 3μM). Vitamin D analogues did not cause any detectable DNA damage in ZR75-1 cells. However, pre-treatment with $1\alpha(OH)D_5$ for 72 hr caused a significant increase in Dox- induced DNA breakage. $1\alpha(OH)D_5$ also enhanced the DNA breakage induced by menadione, an ROS-generating compound, but not that of etoposide, a topo II inhibitor. These results suggest that $1\alpha(OH)D_5$ potentiates the in vitro action of Dox against breast cancer cells. In addition, ROS appear to be involved in the interaction between $1\alpha(OH)D_5$ and Dox. These data may provide a novel treatment option for breast cancer.

529 Capecitabine in Association with Epirubicin (E) and Docetaxel (D) as First Line Chemotherapy in Advanced Breast Cancer: A Dose-Finding Study.

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Capecitabine (Xeloda®) is a rationally designed, oral, tumor-selective fluoropyrimidine that mimics continuous 5-fluorouracil infusion, with good activity in metastatic breast cancer (BC). E and D have both demonstrated high activity in BC: in a phase I study we previous established the safety and maximum tolerated dose (MTD) of ED combination therapy. The main dose-limiting toxicity (DLT) with this combination was febrile neutropenia (FN). We report the results of a phase I study investigating capecitabine (escalating doses: 765-875-985-1,060mg/sqm BID, for 14 consecutive days), in combination with fixed doses of D and E (both administered at 75 mg/sqm, i.v., day 1), every 21 days. Twenty-three patients with metastatic or locally advanced BC were enrolled. Overall, 139 cycles of chemotherapy were given, with a median of 6 cycles per patient. Myelosuppression was the predominant treatment-related adverse event (AE). All patients experienced grade 4 neutropenia and grade 3-4 leukopenia. FN occurred in 6 patients (26%). No grade 3-4 anemia or thrombocytopenia was reported. The majority of non-hematologic AEs were mild to moderate in intensity. Grade 3 AE were rare and there were no grade 4 toxicities. AEs attributed to capecitabine occurred in 5 patients (hand-foot syndrome, nausea and vomiting, diarrhea, mucosal infection, macropapular rash). All were mild (grade 1-2) and resolved without dose reduction or treatment interruption. DLT was reported in all patients treated with the 4th dose level (1 grade 4 afebrile neutropenia and duration >7 days, 1 grade 3 infection, 1 grade 3 mucositis). Objective tumor responses (partial or complete responses) were seen in 21 patients (91%). In conclusion, the recommended dosing schedule for further evaluation has been established as Capecitabine 985mg/sqm, twice daily, 14 days' treatment, with E 75 mg/sqm and D 75 mg/sqm, given i.v. on day 1, (3th dose level). Capecitabine was well tolerated and did not significantly alter the safety profile of ED combination therapy. Moreover, the high response rate observed with this combination regimen merits to be evaluated in phase III trials.

530 Treatment of HER2 Overexpressing Metastatic Breast Cancer (MBC) with Trastuzumab (Herceptin®) and Chemotherapy.

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Response and overall survival of women with HER2 positive MBC are significantly enhanced by a trastuzumab monotherapy or by the combination of trastuzumab/ chemotherapy. Since the FDA-approval of trastuzumab 90 patients with MBC and HER2 overexpression received 117 therapies at our institution between 11/98 and 05/00. Most patients had more than two chemotherapies for MBC. 79% of the patients were pretreated with taxans and/or anthracyclins. All tumors were HER2 positive with a score of 3+ (HercepTest®, DAKO) or a gene amplification of >10 copies (Inform Kit®, Ventana). In 80% of the patients metastases were seen in at least 2 organ systems. Most patients showed visceral (80%), osseous (80%) or cerebral (20%) metastases.

Methods: All Patients received trastuzumab weekly at 2mg/kg, initial 4mg/kg. Additionally, 25 had no chemotherapy, 33 received paclitaxel weekly (80-100 mg/ m2), 23 paclitaxel (175 mg/m2, q3w). Patients with progress under this regimen were further treated: 10 with vinorelbine weekly (25 mg/m2), 17 with capecitabine (2000 mg/m2, d 1-14, q3w), and 9 with docetaxel (75mg/m2, q3w).

Results: median follow up: 10 months

	T	Pw and T	Pqw3 and T	V and T	C and T	D and T
RR(CR,PR,MR)	16%	50%	57%	40%	53%	33%
SD	72%	31%	36%	30%	24%	22%
PD	12%	22%	9%	30%	24%	44%
Median response time	7 (3;12) m	8 (3;12) m	7 (4;38) m	6 (3;9) m	7 (4;17) m	10 (4;17) m
Median TTP	7 (0;16) m	5 (1;10) m	6 (3;9) m	3 (1;5) m	3 (0;8) m	3,5 (2;12) m

T= trastuzumab; P=paclitaxel; V=vinorelbine; C=capecitabine; D=docetaxel; w=weekly; m=months.

Cardiac side effects were seen in 2/117 patients. They were completely reversible after specific therapy. Side effects has been minor for V/T and serious for D/T.

Conclusions: The therapy of the HER2 positive MBC with trastuzumab+/- chemotherapy is effective, well tolerated, and practicable in the out-patient department. Interesting and effective therapy options after progress under T/P are: T/V, T/C, and T/D.

531 A New Second Line Treatment in Advanced Breast Cancer (ABC) Patients: Gemcitabine (G)-Vindesine (V).

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Second line CT has only a palliative aim, so it is very important to select effective drugs with a good toxicity profile. G and V demonstrated clinical efficacy in breast cancer pts but data about activity and toxicity in combination are reported only in lung cancer. We conducted a multicentre Phase II study to evaluate the feasibility and the activity of GV in ABC pts. G was administered at the dose of 1000 mg/mq day 1,8 and V 3 mg/mq day 1, q 21. From March 1999 to May 2000, we treated 33 ABC pts. Median age was 61 years (41-76). The number of metastatic sites was: 1 (18/31 pts), 2 (8/31), 3 (4/31), > 3 (1/31). Visceral metastasis were present in 10/31 pts. 108 cycles were performed: median number per pt: 4 (1-9). All pts but 2 were evaluable for objective responses after 3 cycles. We obtained RP in 8/31 pts (26%) and SD in 5/31 (16%). Main toxicities were described in the Table. Grade III toxicities were: asthenia 15 pts, neutropenia 7. Alopecia G1 occured in 3 pts. No pt discontinued CT. Twenty cycles were 1-week delayed due to haematological (13/20) and hepatic toxicity. This multicentre Phase II study shows that GV is a feasible and well tolerated schedule in heavly pretreated ABC pts.

WHO GRADE	I	II
Nausea and Vomiting	22	4
Anaemia	18	2
Stomatitis	1	0
Cutaneous	1	2
Fever	10	5
Pain	6	9
Leucopenia	21	23
Neutropenia	20	14
Piastrinopenia	4	3
Asthenia	5	18

532 Long-Term Weekly Docetaxel in Breast Cancer (BC) - Safety Analysis.

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Docetaxel (D) is a potent mitotic inhibitor, very active in BC. Weekly administration provides a dose dense schedule that appears a better tolerated option especially in older patients. This study evaluate the feasibility, safety and response of protracted weekly D administration in locally advanced or metastatic BC. D (35 mg/m2) was administered

over 1 hour with dexamethasone premedication 8 mg orally 12 hours before and after and 8 mg iv just prior to administration. It was planned to deliver 4 weeks of treatment as a cycle immediately followed by 4 more weeks unless progression or grade 3 side effects. Of the first 9 patients treated this way, G2/3 neutropenia was fairly common and 3 pts experienced grade 3 fatigue/asthenia. Four pts developed fluid retention G 1 (pleural/pericardial effusions) at 455, 490, 665 and 700 mg/m2 cumulative doses: 79% of the treatment could be given on schedule.

Because of this we replaced the 4th week of D with a week of rest. Overall 15 pts have been treated. Median age 70(56-80); ECOG PS o: 4 pts, 1: 11 pts, stage IIIb: 4 pts, metastatic : 11 pts. A median of 14 weekly doses (5-24) were delivered. Anemia G1 : 8 pts, G2: 5 pts, neutropenia G2/3: 8 pts. No thrombocitopenia or febrile neutropenia was observed. Nail changes in 7 pts and hyperlacrimation in 7 pts. In pts with the week of rest, no fatigue/asthenia G1 and no fluid retention were observed, with 85% of the planned doses delivered. In this small number of pts we observed a progressive decline in total protein (Pr) and albumin (Al) that did not go below the lower limit of normal but was statistically significant difference from baseline:

Cumulative Docetaxel Dose
Baseline 280 mg/m2 560 mg/m2 700 mg/m2
(n:15) (n:11) (n:6) (n:3)
Pr(g/%) 6,97(±0,45) 6,31(±0,5) 5,66(±0,3) 5,40(±0,3) P:0,05
Al(g/%) 3,78(±0,3) 3,39(±0,7) 3,02(±0,2) 2,8 (±0,2) P:0,05

Further exploration of this observation is warranted. Weekly D is a well tolerated regimen (including older than 70) without grade 4 neutropenia or neutropenic complications. A cycle of 3 weeks of treatment, followed by 1 week without, improves tolerability. Efficacy and safety results in additional pts (to be presented) will further clarify the risk/benefit ratio of this palliative regimen in BC.

533 The Association of Taxol and Taxotere Is Feasible and Active for Anthracycline Pretreated Metastatic Breast Cancer: Final Results of a Phase II Study.

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Taxol and Taxotere have a similar chemical root, however, the presence of important biological and clinical differencies may represent the rationale for the co-administration of these two drugs. With these premises, we designed a pilot study on the association of Taxol plus Taxotere in advanced breast cancer. At present 21 out of the 25 planned patients have been enrolled. Two different schedules of treatment have been used: A) Taxol (1 hr) 60/50 mg/sqm days 1&8 + Taxotere 60/50 mg/sqm day 1, q.3 weeks and B) Taxol (1 hr) 40 mg/sqm days 1&8 + Taxotere 25 mg/sqm days 1&8, q.3 wks, up to 6 courses. Patients in response or stable disease received maintenance treatment with Taxol (80 mg/sqm/weekly, 1 hr) until progression or toxicity. Median age was 55 yrs (range 34-70), all patients received prior chemotherapy: 6 only adjuvant, 4 adjuvant + 1 line mts, 6 adjuvant + >2 lines mts. All patients received prior anthracyclines, of these 17 pts for mts disease. 15 patients received prior taxanes: of these 9 taxol, 1 taxotere, 5 both. Dominant metastatic sites were viscera in 13 patients, soft 6 and bone 2; 11 patients had 2 or more involved sites. 16 patients are evaluable for toxicity: grade 3/4 neutropenia was present in 16%; 6% of patients had grade 2 peripheral neuropathy and 31% grade 2 mucositis. Other toxicities included mild to moderate fluid retention and myalgia; no grade 3/4 non hematological toxicities, including alopecia, were observed. At present 15 pts are evaluable for response: overall RR was 80%, CR 20%, SD 7%, PD 13%. The final results from this pilot study will be presented.

534 Pharmacokinetics of Herceptin® Administered with Paclitaxel Every Three Weeks.

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Herceptin® is a humanised monoclonal antobody which, in combination with paclitaxel, has been shown to produce survival benefit in the treatment of HER2-overexpressing metastatic breast cancer. Currently, Herceptin® is administered to patients weekly. However, a less frequent administration may increase patient convenience, especially, those receiving longer term adjuvant therapy.

This study was designed to investigate the pharmacokinetics of Herceptin® and paclitaxel when administered intravenously every 3 weeks. Since preclinical data suggest that a serum level of 10-20µg/mL is required to produce maximum inhibition of tumor growth, this has been chosen as a target level for the comparison of new regimens. Therefore, trough serum concentrations of Herceptin® are measured to predict potential efficacy.

Patients receive Herceptin® initially at a dose of 8mg/kg i.v followed by 6mg/kg i.v. every 3 weeks thereafter. Patients also receive paclitaxel (175mg/m_2) every 3 weeks until week 24 (cycle 8) at which point Herceptin® is adminstered alone. Full pharmacokinetic profiling occurs at cycle 4 and when Herceptin® is administered alone (cycle 12). Trough (predose) and peak (end of infusion) samples are collected for assessment of Herceptin® at each cycle.

To date, a total of 16 patients have been recruited into the study and, of these, 12 are ongoing. Thirteen patients are evaluable for safety. Four have experienced serious adverse events; one with febrile neutropenia, one with pseudomembranous colitis and two with infusion-related reactions. One patient has withdrawn from the study due to adverse events. However, this regimen appears tolerable. Pharmacokinetic data are available for nine evaluable patients, having a mean trough Herceptin® serum concentration of 55µg/mL. Analysis of further Herceptin® and paclitaxel data is ongoing.

Preliminary data compare favourably with those obtained during weekly administration of Herceptin® with trough serum concentrations in the range associated with efficacy being achieved. Pharmacokinetic parameters for Herceptin® and paclitaxel and the assessment of a possible interaction will be presented.

535 Efficacy and Safety Profile of Capecitabine (Xeloda ®) in Combination with Paclitaxel (P) in Patients with Locally Advanced or Metastatic Breast Cancer: Preliminary Results of a Phase II Study.

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Background and methods: The oral fluopyrimidine capecitabine (C) is converted to 5-FU by thymidine phosphorylase, an enzyme with significantly higher activity in tumor compared with healthy tissue. C+P combination therapy has shown synergistic activity in preclinical studies and a phase I trial identified an appropriate dose for further evaluation. We conducted a phase II study to evaluate the efficacy and tolerability of C+P. Pts received intermittent twice daily C 1000mg/m2 (2 weeks (w) on treatment, 1w off) and P 175mg/m2, day 1 q3w. All pts had locally advanced or metastatic measurable BC and had received previous anthracycline treatment (including adjuvant therapy). Toxicity was graded as mild, moderate or severe.

Results: 64 pts treated in 13 centers are currently evaluable for response. The median age of pts was 51 (34-75) years, with a median KPS of 90% (60-100%).77% had 2 or more involved sites. Pts received a median of 7 cycles. The overall response rate in evaluable pts was 62.5% (95% CI: 49.5-74.3%), including 14 complete responses. In a subpopulation of 24 pts treated with adjuvant anthracyclines who received C+P as first line therapy, the response rate was 70.8%. Four of the 8 docetaxel-pretreated pts had partial response. Median time to response was 10.7w (95% CI: 6.0-11.9w), with a median duration of response of 33.1w. The median time to progression was 37.0w (95% CI: 31.3-41.3w). The most common severe toxicities in 66 pts evaluable for safety were alopecia (6 pts), hand-foot syndrome (4 pts), neutropenia (4 pts), febrile neutropenia (2 pts) and nausea/vomiting (2 pts).

Conclusion: In conclusion, C+P combination therapy is a highly effective treatment for locally advanced/metastatic BC (62.5% RR) with an acceptable and predictable safety profile. The study is ongoing and updated results will be presented at the meeting.

536 Escalating Doses of Docetaxel (D) and Epirubicin (E) as First Line Therapy for Metastatic Breast Cancer (MBC). A Phase I/II Study of the National Cancer Institute of Canada - Clinical Trials Group.

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A phase I/II study of increasing doses of D and E was undertaken to determine the toxicity, efficacy and pharmacokinetics of D+E for first line treatment of MBC. Escalation began from the initial dose levels of D 60 mg/m^2 and E 60 mg/m^2 given IV every 3 weeks. Left ventricular ejection fraction was evaluated at baseline and every 2 cycles throughout treatment. The maximum tolerated dose (MTD) was reached at D 75 mg/m^2 and E 75 mg/m^2 with and without cipro prophylaxis for febrile neutropenia (FN). The phase II portion was conducted nationwide at the MTD-1 level: E 60 mg/m^2 and D 75 mg/m^2.

As of March 27, 2000, 58 patients were enrolled; 33 in the phase II portion. Median age was 57 yrs; 28 had ≥3 sites of disease; 25 received adjuvant chemotherapy (3 with doxorubicin, 1 with E); 13 received adjuvant hormone therapy (HT); and 23 metastatic HT; 51 were evaluable for toxicity. The most common hematologic toxicity was grade 4 granulocytopenia in all phase I patients without G-CSF, 7/10 with G-CSF and 20/25 in phase II. Thirteen episodes of FN were reported (in 12 patients), 10 in phase II. The most common non-hematologic grade 3/4 toxicities (n=) were: lethargy (10), pain (9), diarrhea (5), nausea (4), SOB (3), edema (2). CHF was reported in 1 patient; and 1 death was due to disease progression plus treatment toxicity.

The phase I portion continues at E 90 mg/m^2, D 75 mg/m^2 + G-CSF. Pharmacokinetics were performed in cycles 1 and 2 in a subset of phase I patients. In a preliminary report (ASCO abstract #443, 1999) response rates of 63%, phase I, n=23; and 78%, phase II, n=16; were described. The final results including response rates, duration of response, toxicity and pharmacokinetics will be presented.

537 Phase I Study of Weekly Docetaxel in Combination with Capecitabine in Patients with Solid Malignancies.

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Capecitabine is an oral fluoropyrimidine which undergoes conversion into its active metabolite 5-FU by a three-step enzymatic process. The final step is mediated by thymidine phosphorylase (dThdPase), which is preferentially expressed in tumor tissue. Based on the preclinical observation of upregulation of dThdPase by docetaxel, we conducted a phase I study of docetaxel in combination with capecitabine. Since docetaxel-mediated dThdPase upregulation is transient, with maximal activity observed between 4 and 14 days after treatment, docetaxel was administered weekly (days 1, 8 and 15), every 4 weeks, and capecitabine in two daily oral doses was started on day 5 of every course and continued for a total of 14 days (days 5 to 18). Fifteen patients have received 60 courses of docetaxel 36 mg/m2/week and capecitabine 1250 or 1500 mg/m2/d. Dose-limiting toxicities (DLTs), which include grade 3 hand foot syndrome (HFS) (2 patients) and grade 3 diarrhea (1 patient) were observed in 3/3 patients treated at the 36/1500 docetaxel/capecitabine dose level during either the first or second course. Twelve patients (46 courses) were treated at the 36/1250 docetaxel/capecitabine dose level. At this dose, one patient developed grade 3 emesis during the first course and two patients developed grade 3 HFS after 2 courses. An additional patient developed grade 3 asthenia after 4 courses. Other grade 1-2 toxicities observed include onycolysis, diarrhea, pedal edema, pleural effusion and lacrimation. No grade 3-4 hematologic toxicities were observed. The median number of courses administered thus far at the 36/1250 dose level is 3 (range 1-8). Antitumor responses has been observed in patients with paclitaxel-refractory breast cancer (1 partial, 1 minor) bronchioloalveolar carcinoma (1 partial), colorectal cancer (2 partial), cisplatin/gemcitabine-, paclitaxel/carboplatin- and CMV-refractory bladder cancer (1 partial), and hepatocellular carcinoma (1 minor). In conclusion, capecitabine 1250 mg/m2/d for 14 days can be safely administered in combination with 3 weeks of weekly docetaxel 36 mg/m2 every 4 weeks. The preliminary antitumor activity observed encourages further evaluation of this regimen in efficacy trials.

538 A Phase I Study of Cyclophosphamide, Doxorubicin (Adriamycin) and 5-FU/Eniluracil (CAFE) in Women with Advanced Breast Cancer.

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With the availability of new, effective, oral fluoropyrimidines, the potential for the development of more effective doxorubicin-based combination regimens has emerged. We designed a Phase I study to determine the maximum tolerated dose (MTD) of Eniluracil/5-FU, in combination with cyclophosphamide and doxorubicin, in patients with advanced breast cancer. Eligibility includes histologically confirmed locally advanced or metastatic breast cancer, < 3 prior chemotherapeutic regimens in the metastatic setting, adequate bone marrow, hepatic and renal function, and < 360 mg/m2 of prior doxorubicin. Cyclophosphamide (C)and doxorubicin (A)are administered IV on day 1 and oral Eniluracil/5-FU BID on days 1-14, every 21 days. Patients are treated for 6 cycles unless dose-limiting toxicity (DLT) or disease progression intervenes. To date, a total of 72 cycles (range 1-6) have been administered to 19 patients. Full dose cyclophosphamide and doxorubicin (600 mg/m2 and 60 mg/m2, respectively) have been administered in conjunction with the phase II dose of Eniluracil/5-FU (10 mg/m2/1.0 mg/m2 BID) at dose level 4 without dose-limiting toxicity. Dose escalation above these standard doses was not deemed appropriate. Enrollment continues at dose level 4 for validation and confirmation of safety. The principal toxicities have been, not unexpectedly, myelosuppression and mild gastrointestinal toxicities. Responses have been seen in 10 of 15 evaluable patients. Experience to date suggests this regimen is active and well-tolerated, deserving further evaluation in a phase II setting.

539 Phase I Study of Vinorelbine (V) and Capecitabine (C) in Advanced Breast Cancer (ABC).

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The aims of this phase I study were to determine the maximum tolerated dose (MTD), to evaluate the toxicities and to seek the evidence of antitumor activity of the oral fluoropyrimidine C in combination with V. Thirty-three pts, with ABC were included; all pts were evaluable for toxicity and 29 for efficacy. Pts characteristics were: median age 52 yrs (35-71); PS 0/1: 17/12; 26 pts were pretreated for metastatic disease; the most common sites of metastatic disease were liver (17 pts), bone (12 pts), lung(11 pts). C was administered twice daily for 14 consecutive days in combination with V administered i.v. on day 1 and 3 every 21 days at eight levels of dose. Two hundred-seven cycles were administered (range 1-17); only one episode of fever associated with grade(G)4 neutropenia (DLT) was observed during 1 cycle at the 6th level. Neutropenia G3-4 was observed in 67 cycles (32%) and G3 anemia was observed in one cycle. Non-hematological toxicites included asthenia (G3 in one cycle),injection-site reaction (G3 in 10 cycles), increased bilirubin (G3 in 2 cycles) and diarrhea (G3 in one cycle and G4 in one). PR was observed in 14 pts (48%), 1 pt had a CR(4%); 3 pts had a MR (10%) and 6 SD (21%), while 5 progressed on treatment (21%). TTP was 5.0 months (range 2.1-9) The efficacy of the regimen was observed at all dose level. After the inclusion of 33 patients (8th level) the MTD was not reached. Escalating the doses to find an MTD might not be the best strategy for the development of this combination.

dose level	capecitabine mg/m2/day	vinorelbine mg/m2	pts	CR	PR	MR	SD	PD	NE
1	1000	12.5	3	-	-	1	-	2	-
2	1250	12.5	3	-	1	-	1	1	-
3	1250	16.5	3	-	1	-	1	1	-
4	1650	16.5	4	-	1	1	2	-	-
5	2000	16.5	3	1	2	-	-	-	-
6	2000	20	7	-	2	1	2	1	1
7	2250	20	3	-	3	-	-	-	-
8	2500	20	7	-	4	-	-	-	3
total			33	1	14	3	6	5	4

540 Long-Term Follow-Up Results of Metastatic Breast Cancer Patients Treated with S-1 (a New Generation of UFT).

Saeki T, Takashima S, Horikoshi N, Sano M, Kimura M, Miura S, Morimoto K, Noguchi S, Taguchi T, the Breast Working Group, and the S-1 Cooperative Study Group. Tokyo, Japan.

S-1 is a newly generated fluoropyrimidines consisting of 5-fuluorouracil prodrug and reversible dihydropyrimidine dehydrogenase (DPD) inhibitor. We previously reported the efficacy and toxicity of S-1 in a phase II study for the metastatic breast cancer patients (ASCO: 2000, #404). In the present study, we analyzed the compliance of S-1 and survival of 111 breast cancer patients, who were enrolled onto this trial. 40mg/m2 of S-1 were administered twice daily for 28 consecutive days with 14 days rest during one course were repeated every 6 weeks unless progression. In 108 patients who were evaluable for response, there were 10 complete (CR) and 35 partial response (PR) with overall response rate of 41.7% (95% confidence interval (CI), 32.3%-51.5%). Symptomatic and hematological acute toxicity more than grade 3 occurred occasionally, in addition, no treatment related death was observed. The adverse events over grade 3 were noted as follows; neutropenia 9.1% (10/110), anorexia 3.6% (4/110), fatigue 2.7% (3/110), stomatitis 1.8% (2/110), nausea/vomiting 1.8% (2/110), diarrhea 0.9% (1/110), anemia 0.9% (1/110). The median follow-up period for patients was 671.5 days. The median survival time was 834 days (95% CI, 572-1081 days). In addition, the 2-year survival rate was 55.0%. The compliance of each courses was 70.4-80.8%. S-1 may be effective to improve the survival and this schedule of S-1 administration will be tolerable.

541 Inositol Hexaphosphate (IP6) Enhances Growth Inhibition of Breast Cancer Cells by Tamoxifen (TMX) and Adriamycin (ADR).

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The ubiquitous carbohydrate IP6 has anti-neoplastic effects against various types of cancer, including breast carcinomas. IP6 has also been shown to prevent breast cancer in animal models. ADR and TMX are two most important agents in breast cancer treatment, but some patients do not respond to them, thus the quest for better regimens. In this study, we investigated the effects of IP6, alone and in combination with ADR or TMX, against several types of human breast cancer cell lines. Growth inhibition of the estrogen receptor-positive MCF-7, estrogen receptor-negative MDA-MB 231, and ADR-resistant MCF-7 (MCF-7/ADR) human breast cancer cell lines were studied using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. We showed that MCF-7/ADR was the most sensitive cell line (IC50 = 0.95 mM vs. 1.33 mM for MDA-MB 231 and 4.18 mM for MCF-7). When IP6 and ADR were sequentially administered 72 hours after each other, growth suppression of MDA-MB 231 and MCF-7 was even more pronounced (synergistic effects as calculated by Webb's fraction method, $p<0.0001$). Synergism was also observed with the IP6 and TMX combination for all of the cell lines regardless of estrogen receptor status ($p<0.0001$ for MCF-7/ADR). Fresh breast tissue samples that were ADR-resistant also responded to IP6 treatment (IC50 values ranged from 0.91 to 5.75 mM). Our data reproducibly showed the potent anti-neoplastic function of IP6 against different types of breast cancer cells including, the drug resistant ones, thereby holding promise for improving the outcome of treatment for breast cancer.

542 Antisense Oligonucleotide Therapeutics as a New Approach to Breast Cancer Therapy: *In Vitro* and *In Vivo* Studies.

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Since the concept of antisense therapy was introduced over 20 years ago, antisense oligonucleotides have been shown to be unique drugs that achieve their effect by targeting mRNA with which they can hybridize and specifically block protein expression. Thus, antisense drugs offer the possibility of specific, rational, genetic-based therapeutics. In our laboratory, we have been investigating the potential use of antisense oligonucleotide therapeutics in the treatment of breast cancer. Two molecular targets were chosen in the study: cAMP-dependent protein kinase (PKA) and MDM2 oncogene. PKA and MDM2 oncogene have been shown to be amplified or overexpressed in human breast cancer. The mixed backbone oligonucleotides (MBOs) were employed in the study. We designed several antisense oligonucleotides that specifically inhibit PKA or MDM2 expression *in vitro* and *in vivo*. Dose-dependent, sequence-specific *in vitro* antitumor activity was shown in MCF-7 and MDA-MB-468 cells. Nude mice and SCID mice bearing human breast cancer MDA-MB-468 xenografts were treated with antisense MBOs alone or in combination with conventional cancer chemotherapeutic agents, demonstrating significant tumor growth inhibition in a dose- and sequence-dependent manner. These studies strongly indicate that antisense oligonucleotides targeting to PKA or MDM2 oncogene can be used as a new approach to breast cancer therapy. (Supported by NIH/NCI grant CA 80698.)

543 Phase I-II Trial in Locally Advanced Breast Cancer (LABC) with Increased Dose of Continuous Orzel (UFT + Folinic Acid) in Combination with Doxorubicin (D) and Paclitaxel (P) Every Three Weeks.

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Purpose: The aim of the study is to determine the maximum tolerated dose of Orzel (combination of UFT + Calcium Folinate) plus fixed dose of D+P in patients (pt) with LABC. Secondary, clinically and pathological response rate and time to progression were assessed.

Patients and methods: From July 1999 to February 2000, 13 pt with stage IIIB breast cancer were included into the study. D 60 mg/m2 iv bolus & P 200 mg/m2 iv 3 hour infusion were given every 3 weeks. Continuous Orzel (dose levels: 1= 200, 2 = 250, 3 = 300, 4 = 350 mg/m2) were given divided in two daily dose. 4 courses were scheduled and clinical assessment for response was made after 2nd and 4th courses. Surgery was performed if clinical response existed. 3 pt were included in each level and it was increased unless dose limiting toxicity (DLT) (G4 anemia or thrombopenia, febrile neutropenia, other G3 toxicity or G2 neuropathy or cardiopathy) appeared.

Results: 13 pt have been treated and 12 are valuable for clinical response. 47 courses (c) were given (median 4c/pt). Characteristics: median age 49 years (35-63); premenopausal status= 9 pt; ECOG 0= 13 pt; ductal histology 13 pt; T3N2 = 3 pt; T4N0-2 = 10 pt (including 4 T4d).

No DLT appeared in level 1 & 3. One pt developed G3 diarrhea in level 2 and another pt presented neutropenic fever and G4 diarrhea in level 4. Other toxicity: G2: mucositis 4 pt, arthralgia-mialgia 5pt, asthenia 4 pt; hand - food sdr. 1 pt; diarrhea 1 pt; G3: alopecia 13 pt, vomiting 4 pt; G4: neutropenia 9 pt (at nadir). No clinical cardiotoxicity. Clinical response: complete 4 pt, partial 8 pt (100% objective response). 10 pt were submitted to surgery (9 mastectomy and 1 lumpectomy). 1/10 (10%) had pathological response. 10 pt presented understaging.

Conclusions: DLT have not been reached at 300 mg/m2 level. A high clinical activity have been shown and the study is ongoing (updated result will be presented).

544 A Phase II Pharmacokinetic and Pharmacodynamic Trial of 9-Nitrocamptothecin (9-NC) in Patients (pts.) with Metastatic Breast Cancer.

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Background: Camptothecins are a unique class of chemotherapeutic agents which inhibit topoisomerase I activity. Cytotoxicity is S-phase specific and enhanced by prolonged exposure rather than intermittent treatment with high drug concentrations. 9-NC is an orally available camptothecin analogue ideally suited to chronic administration. We report the first phase II trial of 9-NC in pts. with metastatic breast cancer.

Methods: Pts. with previously treated metastatic breast cancer received 9-NC 1.5mg/m^2 once daily for 5 consecutive days followed by a 2 day rest period.

Results: Between 9/99-5/00, 18 pts. were enrolled. Median age was 46.5 yrs. (range 31-73). ECOG PS was 0 in eight pts, 1 in 10 pts. Nine pts. had received 1 prior chemotherapy regimens; 9 pts had received 2 prior regimens. 14 pts. had predominantly visceral disease. Therapy was generally well tolerated; grade IV toxicity was observed. Myelosuppression was mild with grade III neutropenia in only one pt. Grade III nausea or diarrhea were experienced by 3 pts. each. Chemical cystitis was the most common nonhematologic toxicity with 4 pts. experiencing grade III bladder spasms. Dose reductions were required in 4 pts. (2 pts. required 2 dose reductions). Sixteen pts. are evaluable for response; 2 pts. are too early. No objective responses have been seen; overall median time to treatment failure was 45 days. Two pts. have had minor responses (25-49% decrease); an additional 3 pts. have had stable disease with a median duration of response >203 days (range 68-215). Four responding or stable pts. continue treatment. Pharmacokinetic analyses and correlation of topoisomerase levels with response and toxicity are ongoing.

Conclusions: 9-NC was well tolerated but had minimal activity in pts. with previously treated metastatic breast cancer.

545 Phase I Study of Eniluracil (E) Plus Oral 5-Fluorouracil (5-FU) in Combination with Docetaxel (T) for the Treatment of Pateints with Metastatic Breast Cancer: Preliminary Results.

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E is a effective inactivator of dihydropyrimidine dehydrogenase, the rate-limiting enzyme in 5-FU catabolism. E increases the oral bioavailability and plasma t1/2 and reduces the pharmacokinetic variability of oral 5-FU. E /5-FU has antitumor activity in breast cancer. T is considered one of the most active drugs in the treatment of this disease. A phase I study was designed to determine the MTD of E/5-FU with T. Patients had measurable or evaluable disease, Zubrod < 1, and have received no more than 1 prior chemotherapy regimen for metastatic disease. Patients were required to have failed anthracycline-based therapy and never been exposed to taxanes. Patients received oral E/5-FU bid at a dose of 11.5/1.15 mg/m2 or 10.0/1.0 mg/m2 for the first 14 days of each 3 week course. T was given at a starting dose of 50 mg/m2 i.v. over 1 hr on day 1. The dose of T was increased for successive cohorts by 20% until DLT occurred in > 2 pts in a cohort. Eighteen pts have been treated in 4 different cohorts (median age= 52, median Zubrod= 1). A total of 127 courses have been administered. Neutropenic fever and/or infection was observed in 3 of 6 pts in cohort 2 (T=60; E/5-FU=11.5/1.15 mg/m2) and in 4 of 6 pts in cohort 3 (T=72; E/5-FU=11.5/ 1.15 mg/m2). All but two episode occurred after course 1. Other grade 3/4 toxicity included asthenia, diarrhea, and excessive lacrimation. Significant fluid retention has not been observed. Four pts are still on study. Seventeen pts have had a decrease or improvement of their disease with resolution of symptoms and in some instances a decrease in tumor markers. Patient compliance with the oral medication has been good. T in combination with E/5-FU is an active regimen and well tolerated. DLT consists of neutropenic fever which occurred typically at course 3 or later. The recommended phase II dose is 72 mg/m2 of docetaxel and 10.0/1.0 mg/m2 of E/5-FU.

546 Male Breast Carcinomas Do Not Show Amplification of the HER-2/*neu* Gene.

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In 1995, we reported that HER-2/*neu* protein was over-expressed in 35% of male breast cancers. (Int J Surg Path, 1195; 2:199-206). In that study, tumor cells showing moderate cytoplasmic and/or membranous immunostaining were called positive. Other studies assessing HER-2/*neu* status in male breast carcinoma showed similar results but failed to correlate with disease outcome.

We retrieved unstained slides from 61 invasive male breast carcinomas. Three of the cases did not contain residual tumor on the unstained slides and were excluded from the study. The men in this study ranged in age from 38 years to 92 years, (mean 63 years); 35 (60%) were T1 lesions and 23 (40%) were T2 lesions. Twenty-five patients (43%) had positive lymph nodes.

Each case was assessed for HER-2/*neu* protein over-expression by immunohistochemistry (IHC). Slides were immunostained with CB11 (Ventana Medical Systems, Tucson Arizona), a monoclonal antibody directed at the internal domain of the HER-2/*neu* receptor protein. Scoring was performed by light microscopy. Only membranous immunostaining was assessed and cases were called positive if complete membranous immunostaining was seen in more than 10% of the invasive tumor cells. Each case was also assessed for HER-2/*neu* gene amplification by Flourescence in-situ hybridization (FISH). The PathVysion assay (Vysis Corporation, Downers Grove, Il) was used and scored as positive if the ratio of the number of copies of the HER-2/neu gene to the centromeric probe for chromosome 17 was greater than 1.8.

One (1.7%) of the 58 cases showed 3+ over-expression of HER-2/*neu* protein. Of the 57 cases that did not show over-expression, 43 (74%) were assessed as 0+ and 14 (24%) were assessed as 1+. There was no amplification of the HER-2/*neu* gene in any of the 58 cases. The case, which was 3+ positive by IHC, was re-assessed for gene amplification a second time and the lack of gene amplification was confirmed. It appears that male breast carcinomas do not show evidence of HER-2/*neu* gene amplification. Cases showing over-expression of HER-2/*neu* protein without amplification of the HER-2/*neu* gene, the so-called single copy over-expressers, appear to occur with a frequency similar to that seen in female breast cancer.

547 Patterns of Body Size in Male Breast Cancer and Impact of Obesity on Disease Outcome - The Toronto Experience.

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BACKGROUND: Carcinoma of the male breast is rare, although several similarities exist with female breast cancer (BC) and in recent years management principles of the latter have been extended to treatment of the disease in males. Obesity has been identified as a risk factor and adverse prognostic factor in female BC. Among Canadian adults, 37% of women and 59% of men are obese (body mass index = weight in kg/ (height in m)^2 of ≥ 25kg/m^2). At the Toronto-Sunnybrook Regional Cancer Centre (TSRCC), 54% of women with BC receiving systemic therapy have a BMI ≥25. Patterns of body size in the male BC population are less well defined, as is the role of obesity in the prognosis of the disease. We sought to determine the distribution of body size and its impact on outcome in the pooled male BC population of the two tertiary cancer centres in Toronto; the Princess Margaret Hospital (PMH) and the TSRCC.

METHODS: The medical records of all men presenting with BC between 1955-1996 to PMH and 1980-1999 to TSRCC were reviewed. Recorded height and weight were used to calculate body surface area (BSA) and body mass index (BMI).

RESULTS: This series includes a total of 287 men, with complete anthropometric and follow-up data available for 203. Mean age at diagnosis was 62.2 ± 12.24 years. Mean height was 171.8 ± 8.07cm and mean weight was 79.5 ± 12.77kg. Mean BSA was 1.9 ± 0.16m^2 and 27% had a BSA ≥ 2m^2. Mean BMI was 27 ± 4.08kg/m^2 and 67% had a BMI ≥ 25kg/m^2. Nearly half (48%) were mildly obese (BMI 25-29.9), 20% were moderately so (BMI 30-39.9) and none were severely obese (BMI ≥ 40). At a median follow-up of 143.4 months, median survival is 85 months with 95%C.I. (68.4-97.7 months). A survival analysis on the basis of body size revealed no deleterious effect of BMI ≥ 25 on overall survival, although there were more events in this obese group.

CONCLUSIONS: Obesity is prevalent in this series of men with breast cancer, more so than among their female counterparts, and the general male population. Although our sample size is limited, obesity does not appear to have an adverse impact on outcome in this population. The impact of body size within traditional prognostic subgroups remains under study.

548 Carcinoma In Situ of the Breast in Males and Subsequent Invasive Breast Cancer.

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Male breast cancer is rare, and scant information is available for the subgroup of patients having carcinoma in situ (CIS) of the breast. The SEER Cancer Incidence Public-Use Database (1973 to 1996) recorded 164 male patients with primary CIS of the breast. Only diagnoses with recorded pathology were included, and diagnoses made at the time of death were excluded to ensure follow-up of subsequent cancers. The development of invasive breast cancer was analyzed by Kaplan-Meier estimation. The median follow-up was 5.8 yrears, and the median age at diagnosis was 63 years. One hundred and sixty three patients (99.4%) were treated with mastectomy, and 9 patients (5.5%) received radiation therapy. Pathology included ductal CIS (88.4%), lobular CIS (1.8%), other CIS (9.8%). Twenty patients developed 25 subsequent cancers, and 2 of these were invasive breast cancer. Three additional cases were identified by only the ICD code for death attributed to male breast cancer, and these patients were excluded due to the lack of documented pathology. The 2 cases of subsequent invasive breast cancer occurred in the contralateral breast after 12.5 and 16.8 years of follow-up. The actuarial rate of subsequent invasive breast cancer was 3.1% at 15 years. CIS of the male breast appeared to be a marker for subsequent invasive breast cancer although the magnitude of risk appeared to be smaller than CIS of female breast. Further follow-up is needed to evaluate the long-term outcome in males with CIS breast disease.

549 Men with Breast Cancer Have a Better Disease-Specific Survival When Compared to Women.

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Introduction: It is thought that, women and men with breast cancer have similar survival. This study estimates and compares the disease-specific and overall survival of male and female breast cancer patients matched for age, stage, who were treated in the same medical center within the same time frame.

Methods: Each man in the prospective breast cancer database at Columbia Presbyterian Medical Center between the year 1980 and 1998 was matched with a woman from the same database. Matching was done based on age at diagnosis (within one year), date of diagnosis (within 7 years), stage and histology of the primary.

Results: A total of 52 out of 55 male breast cancer patients could be matched. The median age at presentation was 70 years (range 37-90) for women and 70 years (range 38-91) for men. Median follow up was 5.2 years for women and 5.5 years for men. Comparing the Kaplan-Meier curves for time to death from breast cancer, there was a significant difference (p=0.05 by logrank test) between men and women. The 5 and 10-year breast specific survival for women were .81 and .7; for men it was .9 and .9. In a Cox regression analysis for time to death from breast cancer, stage was the only predictor of death that approached significance (p=0.057). Sex was not significant after adjusting for other matching factors.

Comparing the Kaplan-Meier curves for overall survival, there was no significant difference (p=0.94 by logrank test) between men and women. The 5 and 10-year overall survival for women were .77 and .51; median survival is 11.6 years. The 5 and 10-year overall survival for men were .77 and .56; median survival is 11.1 years. In a Cox regression for overall survival, age at diagnosis was the only significant predictor of death (p=0.03). Sex was not significant after adjusting for other matching factors.

Conclusion: Men with breast cancer tend to die less frequently of disease when compared to their female counterpart. Both genders had the same overall survival. Stage of disease was the only predictor of death from breast cancer irrespective of the age at diagnosis, sex or type of histology.

550 Estrogen and Progesterone Receptors Comparison in Age-Matched Men and Women with Breast Cancer.

El-Tamer MB, Hibshoosh H, Troxel A, Brenin D, Schnabel F, Ditkoff BA.

Introduction: Estrogen and progesterone receptors (ER & PR) have been reported to be more frequently positive in men than in women with breast cancer. This study reports and compares the ER & PR status of an age-matched group of men and women with breast cancer, who were treated in the same medical center.

Methods: Each man in the prospective breast cancer database at Columbia Presbyterian Medical Center between the year 1980 and 1998 was matched with a woman from the same database. The matching criteria were based on age (within one year), stage and histology. All ER & PR were evaluated using tissue blocks and stained by immuno-histochemistry method.

Results: A total of 55 male breast cancer cases were identified in the database. The median age at presentation among women was 70 years (range 37-90), and 70 years (range 38-91) for men. Median follow up was 4.0 years for women and 5.6 years for men. There were 43 pairs for which ER status was available for both males and females. Eighty-eight percent and 79 % of males and females respectively, had tumors that were ER positive. There was no significant association between sex and ER status after matching (p-value from McNemar's test=0.29).

There were 35 pairs for which PR status was available on both men and women. Of men 74% of the tumors were PR positive, while among women, 71% were PR positive. There was no significant association between sex and PR status after matching (p-value from McNemar's test=0.8).

Conclusion: Men and women with breast cancer have similar incidence of ER and PR positive tumors when matched for age at diagnosis, stage and histology of tumors.

551 Immunohistochemical Characterization of Mammary Carcinomas in Men.

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Carcinoma of the male breast (MBC) is rare and accounts for approximately 0.7% of all breast carcinomas in the U.S. Consequently, the status of tumor markers considered important in disease progression, biologic behavior and response to adjuvant therapy has not been fully evaluated. Using immunohistochemical methods, we have analyzed fourteen cases of MBC (11 invasive, 3 in-situ ductal carcinomas [DCIS]) for the expression of estrogen (ER), progesterone (PR) and androgen (AR) receptor, as well as p53, pRb, HER-2 and Fhit oncogenes. In addition, we have evaluated 5 cases of gynecomastia showing epithelial hyperplasia.

Normal duct epithelium consistently expressed ER, PR, AR, Fhit and pRb, while invasive carcinomas showed decreased expression of ER (7/11), PR(7/11), AR (2/11), pRb (2/11) and Fhit (5/11). P53 was overexpressed in 4/11, while HER-2 was expressed only in one case. In-situ carcinomas were characterized by consistent ER expression, reduced PR, AR, pRb, and a complete loss of Fhit without HER-2 expression. These results showed that MBC has lower frequency of AR expression than female carcinomas. This loss of AR, pRb and Fhit was observed at the level of DCIS, indicating their importance early in the disease progression.

552 Structured Exercise Improves Physical Functioning in Women with Breast Cancer (BC): Results of a Randomized Controlled Trial.

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Introduction: This study evaluated the effect of a self-directed (SD) versus supervised (SUPER) exercise program on health related quality of life (HRQL) in women with BC receiving adjuvant therapy (AT). Secondary outcomes of interest were changes in aerobic capacity (VO_2), and body weight (BW).

Methods: 123 patients with stage I-II BC were recruited, stratified by type of AT (chemotherapy/other), and randomly allocated to one of three groups: usual care (UC), SD, or SUPER exercise. SD and SUPER participants received a progressive walking based program at 50-60% $V0_2$ max. 3-5x/week for 26 weeks performed either at home or the ORCC respectively. Evaluations of generic HRQL (SF-36), VO_2, and BW measures at baseline and 26 weeks were obtained. Changes in SF-36 physical functioning (PF) scores, $V0_2$ and BW between baseline and 26 weeks were compared using one-way ANOVA. Post-hoc analysis was conducted for PF using Dunnett's t-tests (see table).

Results: PF decreased in the UC and increased in the SD and SUPER groups. Supervised exercise improved $V0_2$ (3.5ml/kg/min; p=.01) and reduced BW (-4.8kg p<.05) compared to UC in non-chemotherapy group.

Changes in Physical Functioning (95% confidence Intervals)

Physical Functioning	Usual Care (n=41)	Self-Directed (n=40)	Supervised (n=42)	P value	Post-hoc
All	-4.1 (-11.4,3.3)	5.7 (2.1,9.2)	2.2 (-2.3,6.8)	.04	SD>C(p=.01)
Chemo	-4.9 (-15.4,5.5)	6.3 (1.4,11.2)	.8 (-5.4,7.1)	.11	SD>C(p=.03)
Non-Chemo	-2.1 (-9.9,5.6)	4.3 (-0.2,8.9)	5.0 (-1.5,11.5)	.19	N.S.

Conclusion: Moderate intensity physical exercise can attenuate the reduced physical functioning, improve $V0_2$ and reduce BW; in women receiving AT for BC.

553 Quality of Life in the Anglo-Celtic Randomised Trial of High Dose Adjuvant Chemotherapy.

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Patients with histologically proven operable stage II or IIIa breast cancer with involvement of at least 4 lymph nodes were randomised to receive either doxorubicin (75mg/m^2) followed by conventional dose CMF (cyclophosphamide, methotrexate and 5-fluorouracil) or doxorubicin (75mg/m^2) followed by high-intensity chemotherapy with stem cell support. The two treatment arms had different implications for quality of life (QoL). Patients on the conventional arm of the trial received chemotherapy for a longer period (approximately 9 months compared with 4 on the high dose arm) whereas the high dose treatment arm was much more intense and had an increased risk of short term side effects. QoL was assessed at randomisation, 6 months and 1 year using the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire (QLQ-C30), which is a widely used and validated assessment of QoL. Data were analysed for 166 patients (mean age 43.78 years, range 22-58), 82 on the convential arm and 84 on the high dose arm. There was no significant difference between the groups at the 6 month and 1 year follow-up. At 6 months both of the regimens had resulted in a significant deterioration in QoL, but after a year QoL had returned to a level similar to that reported at baseline. The analysis of the funtional sub-scales of the QLQ-C30 indicated that the high dose treatment had a greater impact on everyday social functioning in the short term, but after a year the data for the two groups were very similar. The trend for emotional function was of interest in that patients on both arms of the trial were significantly less tense and worried at 6 months and 1 year than at baseline.

554 An Informatics System Designed to Assist in Making Breast Cancer Adjuvant Therapy Decisions.

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The goal of the computer program, Adjuvant!, is to allow health professionals and their patients with early breast cancer to make more informed decisions about adjuvant therapy. The program allows physicians to tailor analyses to individual patients and produces user-friendly output that can be shared with patients to help guide adjuvant therapy decisions. Actuarial analysis was used to project outcomes of patients with and without adjuvant therapy based on estimates of prognosis largely derived from SEER data, and estimates of the efficacy of adjuvant therapy based on the 1998 Overviews of randomized trials of adjuvant therapy. These estimates can be refined using a Prognostic Factor Impact Calculator which uses a Bayesian method to make adjustments based on relative risks conferred and prevalence of positive test results. Based on entry of patient information (age, menopausal status, comorbidity estimate) and tumor staging and characteristics (tumor size, number of positive axillary nodes, estrogen receptor status) baseline prognostic estimates are made. Estimates for the efficacy of endocrine therapy (5 years of tamoxifen), and poly-chemotherapy (CMF-like regimens, or anthracycline based therapy, or of therapy based on both an anthracycline and a taxane) can then be used to project outcomes presented in both numerical and graphical formats. Outcomes for OS and DFS and the improvement seen in clinical trials, are reasonably modeled by Adjuvant!. Additional speculative estimates of years of remaining life expectancy and long term survival curves can also be produced. Output printouts of the program have been tailored to be easily interpreted by patients.

Help files supply general information about breast cancer including a discussion of the Overview and its controversial aspects, and the use of prognostic factors. The program's Internet links supply national treatment guidelines, Cooperative Group trial options, and other related information. We have extensively evaluated this tool in patient care settings and found it well accepted by physicians, and their patients. The computer program, Adjuvant!, can play practical and educational roles in clinical settings.

555 Results of a Randomized Trial of a Computerized Decision Aid "Adjuvant!" to Present Tailored Prognostic Information to Stage I-III Breast Cancer Patients.

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We have developed a decision aid to help strengthen the role of the patient in the decision making process. Decision aids can provide the physician with more accurate information to guide decision making. Our decision aid provides a method of estimating the benefit of different adjuvant treatment options for individual patients based on prognostic information. We report on the results of a randomized trial of a decision aid to help newly diagnosed breast cancer patients, in conjunction with their physicians, make adjuvant therapy decisions. A Decision Guide that presents specific prognostic information to breast cancer patients in the form of colored bar graphs has also been developed. These bar graphs are tailored to each woman using our newly developed computer program Adjuvant!. The Adjuvant! program uses a life table analysis technique and factors in natural mortality in addition to excess mortality due to breast cancer. Estimates of breast cancer mortality are based on SEER data. Estimates of treatment efficacy are largely derived from the 1998 meta-analysis of breast cancer adjuvant therapy clinical trials.

A randomized trial of the decision aid was conducted with 400 patients and their medical oncologists (n=45) in two distinct geographic regions, Cleveland, OH, and San Antonio, TX. Physicians were randomized to receive either the output produced by Adjuvant! or no output. Patients received either the individualized Decision Guide produced by the Adjuvant! program or a generic brochure about adjuvant therapy. The presentation will report the results of the clinical trial of Adjuvant! and its impact on: 1) treatment decisions, 2) patient understanding of treatment and prognostic information, 3) patients' immediate satisfaction with the decision, 4) patients'decisional regret. We also present a cost-utility analysis comparing standard decision-making with decision-making aided by the Adjuvant! program and the presentation of the Decision Guide.

556 Surgeons as Counsellors.

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Aim: In the TRACE (Trial of genetic assessment in breast cancer) study, the data on the time spent by the surgeon with the women with a family history of breast cancer was analysed with respect to the changes in the anxiety scores and breast cancer worries scores. We wish to see if spending more time by surgeons improves these scores.

Patient and Methods: In the TRACE study, women with a family history of breast cancer were prospectively randomised to receive a multidisciplinary assessment service (comprising of breast surgeons, nurse specialists and clinical geneticist) in the trial arm, compared with those in the control arm who attended a standard breast clinic to be seen by a surgeon and nurse specialist. Anxiety scores (using the State-Trait Anxiety Inventory) and breast cancer worry scores (using the Breast Cancer Worries Scale) were recorded prior to attending the clinic and immediately after their appointment.

Results: Of the 290 patients in the control group, the time spent by the surgeon was not noted in 16 patients leaving 274 evaluable patients. There was a significant improvement in the anxiety and breast cancer worries after surgical consultation. The surgeons spent between 6 to 55 minutes (mean 22 minutes, SD 7.21). There was no correlation between the length of surgical consultation and reduction of anxiety scores or breast cancer worry scores. There was no difference in scores between individual surgeons.

The patients were then subdivided according to the risk of breast cancer as calculated qualitatively by the breast surgeon into average risk, moderate risk and high risk. In the high risk group was there a significant difference in reduction of breast cancer worry score with longer consultation (p=0.032).

The patients were also stratified according to their preclinic anxiety scores into less worried, moderately worried and very worried. There was a significant reduction in the anxiety scores with respect to length of surgical consultation in the moderately worried group (p= 0.009)

Conclusions: Spending more time with the women with a family history of breast cancer does not help to reduce the anxiety scores and breast cancer worry scores except in the subset of women who were told that their risk of breast cancer is high. If a pre-clinic stratification according to anxiety is feasible, moderately worried women can benefit from a longer consultation.

557 The Effects of a Group Exercise Intervention for Women with Breast Cancer Currently Undergoing Chemotherapy Treatment.

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Fatigue is one of the most common side effects reported by cancer patients undergoing chemotherapy. Exercise has begun to be recognized as an effective treatment for reducing fatigue. Unfortunately, most cancer patients currently receiving chemotherapy do not feel that they have the energy and stamina to participate in any type of physical activity, let alone a regular exercise program. Additionally, physicians are reluctant to encourage patients to engage in exercise despite the potential benefit. The data reported here demonstrate the feasibility and efficacy of a structured exercise group for women with breast cancer, who are currently receiving chemotherapy. Nineteen women with primary breast cancer (stages I-III) participated in a 16 week, 3 times per week, structured exercise group consisting of stretching, aerobic training, and resistance training. Eleven women completed their initial surgical treatment and adjuvent chemotherapy prior to beginning the exercise group, while the other eight women began the exercise group during their chemotherapy regimen. Participants underwent assessments of fitness, mood/distress, and quality of life at intake (baseline), 8 weeks, and 16 weeks. Relative to baseline, participants in both groups experienced increases in fitness and quality of life and decreases in distressed mood. Decreases in reported fatigue were also noted. No significant differences were found between the two groups in the amount of benefit or adherence. These results suggest that breast cancer patients, during any stage of their treatment, can tolerate a structured exercise group and receive significant benefits in a number of areas. Of key clinical concern is the efficacy of treatment for iatrogenic fatigue. This project serves as model for the addition of exercise as adjuvent cancer treatment.

558 Improving Adherence to Breast Cancer Treatment in Public Hospital Patients Presenting with Advanced Disease.

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Background:Women who are from underserved and minority populations(pop)tend to be diagnosed(dx)with breast cancer at later stages of disease(dz)and have increased morbidity and mortality (M&M) stage for stage compared with other pop.One of the factors(fx)that may contribute to the higher M&M may be compliance (compl) with treatment(tx)in this pop.Access to tx may not be enough to overcome the social,cultural and logistic barriers that these patients(pts)face.The theory is that lowering social/logistic barriers can improve compl to tx.Materials and Methods: Pts evaluated for a study of locally advanced breast cancer(LABC) made up two groups(grp).The grp entering the study(A)had per visit support in the form of vouchers for transportation,childcare and groceries.(A)also had contact with the study coordinator.(A)was compared with the pts who were ineligible or chose not to enroll-grp B(B).B did not receive support.The 2 grps were compared for prior health behaviors and time to 1st visit(indicators of chance of compl),for compl with workup(w/u)and chemotherapy(CTX). Delays in CTX were noted as due to medical/system or pt fx. Pt fx were examined.Results:From 1/98-10/99 60pts were evaluated. 47pts(78%)AfricanAmerican,8pts(13%)Latina,4pts(7%)Caucasian and 1pt(1%)Asian.24 had metastatic dz.Of 36 with LABC,7 were ineligible for medical reasons and 5 declined,leaving 24pts in A and 36pts in B. Prior health behaviors were similar in both:seeing a primary care physician-A:12/24pts(50%),B:17/36pts (47%);appropriate mammography history(hx)-A:7/24pts(29%),B:6/36pts(17%),appropriate gyn visit hx: A:13/24pts(54%),B:14/36(39%).Pt report of time that the mass was present-A:average(avr)11.7months (mo)range(r.)0-120mo vs B:10.7mo r.1-48mo.Compl with pre-tx w/u was A:100% vs B:92%. Days(d)from 1st visit to start of CTX was A:32d r.16-94d vs B:avr42d r.12-110d.There were pt fx delays in CTX in A:7/24pts (29%)vs B:14/36pts(39%).In A 1/7(14%)delays were due only to logistic fx(i.e.childcare)vs B:4/14(29%).

Conclusions:Providing support for pts,as well as one on one contact with a care provider can decrease time to start of tx and improve adherence in pts who are medically underserved and at socioeconomic disadvantage. Good compl to tx can be achieved even in a group of pts presenting with late stage disease with a poor hx of health seeking behaviors. Additional studies are needed to show that improving compliance will mean improved survival.

559 Surgery Strategies, Quality of Life, and Conspicuous Psychosocial Constellations in Women with Breast Cancer.

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We questioned to what extent quality of life (QL) in women with breast cancer (BC) is influenced by the outcome of different surgery strategies.

In a retrospective study we evaluated 432 women (response 62%) with primary non-metastatic BC who were treated by mastectomy (ME) (n=93) or breast concerving therapy (BCT) (n=339) between 1991 and 1996. Shoulder-arm morbidity and cosmetic outcome were evaluated by self assessment and physical examination as well. QL was measured with a 59 items self report questionnaire that reliably covered 9 dimensions. Descriptive, test-statistical and latent class analyses (LCA) were done. In both groups shoulder-arm problems were the major complaints followed by fear of progression and dissatisfaction with cosmetic outcome, especially in ME cases. In all QL dimensions we found a varying percentage of women who states negative results, mostly around 20%. But with con-ventional test-statistical means we found no differences between the two surgery groups in almost all QL variables, except of one: BCT patients had significantly less problems with their body image than those who received ME (p=0.003). Patient's age (mean: 51,5 years) or time since surgery (mean: 2,9 years) had no influence on QL. With LCA we could identify 3 conspicuous psychosocial constellations in the total sample: Type 1 (20%) had high scores on anxiety and depression, and low scores on psychosocial resources. Type 2 (16%) had low scores on social support and positive attitudes towards their life. Type 3 (27%) had high scores on dissatisfaction in life, loss of energy, and dissatisfaction with the cosmetic results of surgery. Only 37% stated no problems at all. These types were independent from patient's age , the time since surgery or the surgery strategy.

Data will be discussed under aspects of psychological and surgery related comorbidity, and differential psychooncological support programs for patients with identified psychosocial risk factors in order to optimize treatment efforts.

560 Do Women with Breast Cancer Have Differing Requirements from Hormonal Therapy by Tablet or Injection?

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Purpose - To determine whether patients' survival aspirations changed if treatment was given by tablet or injection.

Method - This preliminary analysis reports interviews with 52 patients in a trial of tamoxifen and/or goserelin (Zoladex) or no treatment for early breast cancer.

Results and discussion - Thirty interviewees (58%) received Zoladex subcutaneous injections monthly and 22 (42%) daily tamoxifen tablets or no treatment. Given a hypothetical survival time without hormonal therapy of 15 years, interviewees required a median minimum survival benefit of a further 5 years from hormonal therapy (n=52). Those who had monthly injections were asked what their required survival benefit would be without injections. Only 3/30 patients (10%) would change their requirements, 2 accepting less benefit from tablets, 1 requiring greater benefit from tablets. Patients who had not had Zoladex were asked what their survival requirement would be from treatment by monthly injection, 4/22 (18%) would require a changed survival benefit from injections, 3 requiring greater benefit, 1 accepting injections for less benefit. No significant differences were found between expected benefits for treatment with or without injections, 45/52 patients (87%) expecting the same improvements from both. Of the 7/52 (13%) with differing expectations, 5 required more from injections and 2 required more from tablets.

Conclusion - Although injections are commonly perceived as less appealing than tablets, only 10% of women in this study felt that a longer survival benefit would be necessary to make injections worthwhile, and 4% would accept less improvement in survival time from injections than tablets. A preference for or against injections was not strong enough to alter survival aspirations of treatment for most women in this study.

561 Is Angiogenesis a Predictor of the Development of Bone Metastasis? Results of Immunohistochemical Study Using CD31 and Factor VIII for Angiogenesis Assessment in Primary Breast Cancer.

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Introduction: Bone is the commonest metastatic site for breast cancer and its destruction is the cause of major and devastating complications. Increasing evidence in the literature suggest a positive correlation between tumour angiogenesis and prognosis. However, to our knowledge there is no study investigating a direct correlation of cancer angiogenesis and established bone metastasis. Our aim was to assess the predictive value of angiogenesis of the primary cancer in the development of bone metastasis.

Methods: Tumoral angiogenesis was assessed in the archival blocks of 167 patients with a histological diagnosis of invasive ductal carcinoma treated surgically at our hospital between 1978 and 1994 followed for a median of five years (Range 3-12 years). Bone metastases were confirmed by isotope bone scans and radiology. Staining was performed with Avidin-Biotin immunoperoxidase technique using two endothelial marker monoclonal antibodies (Von Willebrand factor VIII and CD31). Microvessel Density (MVD)was determined using manual count.Tumoral angiogenesis was correlated with classical clinico-pathological prognosticators: Age, Grade of the tumour, and TNM stage of the cancer.

Results: 89/167 patients had bone metastasis (BM) within 5 years of diagnosis and 78/167 had no bone metastasis (NBM). Ductal carcinoma in situ in the same section was present in 34/167. The mean age in the BM group was 61.5 and 53 in the NBM group. There was a statistically significant difference in the MVD using CD31 and VIII ($P < .01$). There was no significant interobserver variation of the mean count. In univariate analysis there was a significant association between MVD, Grade, lymph node status and the presence of bone metastasis ($P < 0.007$). Patients who developed bone metastasis had higher MVD than those without bone metastasis($P<0.018$). Age and type of operation were not statistically significant ($P = 0.2$). In multivariate analysis, only MVD ($p< 0.008$) and lymph node status ($P<0.02$) remained significantly associated with bone metastasis.

Conclusion: Determination of angiogenesis in primary breast cancer may be useful in identifying patients likely to develop bone metastases and therefore allow selection of patients for prophylactic adjuvant therapy.

562 Angiogenesis in Ductal Carcinoma In Situ (DCIS) of the Breast.

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This study identifies vascular changes associated with progression of ductal carcinoma in situ (DCIS) to invasive carcinoma.

20 sections of pure DCIS were stained with factor VIII, CD31, CD141 and CD34. These were matched for nuclear grade with 20 cases of DCIS with associated invasive carcinoma. In each section, individual foci of DCIS were identified (up to 50/slide) and all vessels within 100 μm counted. The results were expressed as microvessel density (MVD). Normal lobules at least 2 mm away were used as controls.

The highest MVD surrounding normal lobules was obtained using factor VIII antibody, whilst the highest count for pure DCIS and DCIS associated with invasion was obtained using the CD34 antibody. There were significant changes in MVD and vessel phenotype from normal, through pure DCIS to DCIS with invasive carcinoma. The number of vessels staining with factor VIII decreased while the number staining with CD 34 increased. MVD was higher in intermediate nuclear grade DCIS compared to low grade DCIS, but was lowest in high grade DCIS with necrosis.

Angiogenesis in DCIS associated with invasive cancer exhibits an endothelial phenotype that is different from that in pure DCIS. MVD initially increases with nuclear grade but appears to be unable to keep pace with the rate of growth in high grade DCIS.

563 Microvessel Density and Vascular Endothelial Growth Factor (VEGF) Expression in Infiltrating Lobular Mammary Carcinoma.

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Background and Objectives: Angiogenesis is an important prognostic factor in infiltrating ductal carcinoma (IDC). Vascular endothelial growth factor (VEGF) stimulates angiogenesis in vivo. VEGF expression has been correlated with high vascularity in IDC. However, little is known about the prognostic significance of microvessel density (MVD) and its correlation with the expression of VEGF in infiltrating lobular carcinoma (ILC). We analyzed tumor samples from 51 patients with primary classic ILC to determine the relationship between tumoral MVD and VEGF expression. Cases of pleomorphic lobular carcinoma and tubulolobular carcinoma were excluded.

Materials and Methods: Five um sections from formalin-fixed, paraffin embedded tissue blocks were immunostained with antibodies to Factor VIII related antigen (Dako, Carpintera CA) and VEGF (Calbiochem, Boston MA). The former was used for MVD analysis; the vessel counts from the three most vascular fields (0.6941 mm^2, x200 magnification) were recorded and the highest of the vessel count of the three fields was designated as the MVD. The intensity of VEGF staining and the proportion of cells staining were scored. Both the vessel counts and the scoring of VEGF staining were evaluated by two pathologists. The MVD was correlated with the clinical data and the extent of VEGF expression.

Results: There was good correlation between the MVD of each observer (correlation coefficient=0.775, $p<0.001$). The MVDs in patients with stage 3 and 4 disease was higher than those with stage 1 and 2 disease ($p=0.031$ Chi^2 test). However, the MVD was not significantly different between axillary lymph node positive cases and negative cases, between patients with recurrence and those without, and between patients who survived and those who died of disease. There was a very weak negative correlation between the MVD and VEFG expression.

Conclusions: Although the MVD correlated with the stage of disease, it was not predictive of tumor recurrence and patient survival. The absence of a statistical significant correlation between MVD and VEGF expression suggests that other factors may play a more important role in angiogenesis in ILC.

564 Vascular Endothelial Growth Factor: A Surrogate Marker of Response in Neoadjuvant Chemotherapy of Breast Cancer? Preliminary Results of a Clinical Trial.

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Vascular endothelial growth factor (VEGF) is known to stimulate endothelial growth and angiogenesis. The clinical role of VEGF as a prognostic factor is discussed controversially.

In a prospective clinical trial the dynamic course of VEGF was assessed in patients with breast cancer (cT>3cm N0-2 M0) receiving a neoadjuvant chemotherapy (Adriamydin 50 mg/qm, Docetaxel 75 mg/qm, GEPARDO-Study). Sera were collected before each of the 4 cycles of chemotherapy and before surgery, respectively. VEGF was measured by a quantitative enzyme-linked immunosorbend assay.

Between 1/1999 and 10/1999 a total of 100 VEGF levels were assessed in 20 consecutive patients with breast cancer (cT2 n=16; cT3 n=4; cN0 n=15; cN1 n=5). 4 of 20 (20%) patients had a pathological complete response at time of surgery. The other patients (80%) showed residual disease (pT1 n=9; pT2 n=7; pN0 n=10; pN1 n=10).

Before chemotherapy median VEGF level was 280.5 pg/ml (range 30-750) and increased to 483 (188-848) after chemotherapy with statistical significance ($p=0.017$). No significant difference was observed between patients with pathological complete response and patients with residual disease ($p=0.65$).

In addition, a negative correlation between VEGF and haemoglobin content was found with statistical significance after 2 ($p=0.046$) and 3 ($p=0.012$) cycles of chemotherapy. A positive correlation between VEGF and thrombocyte value was observed which reached statistical significance prae ($p=0.009$), after 1 ($p=0.006$), after 2 ($p=0.041$), and after 3 ($p=0.003$) cycles of neoadjuvant chemotherapy.

Our preliminary data suggest that VEGF in serum is not a surrogate marker in this context. Thrombocytes and haemoglobin seem to influence VEGF in sera. Further investigations are necessary to understand the clinical relevance of VEGF.

565 Higher Cytosolic VEGF Content from Ductal Carcinoma In Situ (DCIS) Specimens of the Breast, Than in Benign Adenomas or Invasive Breast Carcinomas: Predicting Increased Risk of Local Relapses?

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VEGF is suggested as the major angiogenic factor in human breast cancer, and we have earlier reported high cytosolic VEGF content in primary breast tumors to be associated with shorter survival times for patients with both node-negative and node-positive breast cancer (J Clin Oncol 1998; 19: 3178- 3183, and J Clin Oncol 2000; 18; 1423-1431).

The aim of the present study was to determine VEGF expression in cytosols from patients undergoing surgery for benign adenomas or DCIS, and to compare the VEGF content with the VEGF content in patients with invasive breast carcinomas. Secondary aims were to determine the possible prognostic value of VEGF for patients with DCIS, in relation to therapy given; modified radical mastectomy, breast conservative surgery with or without radiotherapy.

A total of 106 patients (60 with benign adenomas and 46 with DCIS) were included. The VEGF content in specimens from DCIS was significantly higher than in benign adenomas (median 4.14 pg/µg DNA; range 0.11-68.0, and 1.42 pg/µg DNA; range 0.0-46.0).

VEGF content in DCIS was also higher, than in invasive breast carcinomas (median 2.40 pg/µg DNA; range 0.11-144.78), although not statistically significant.

The results indicate that VEGF expression might be a critical factor early in carcinogenesis. The possible prognostic potential will also be presented.

566 Vascular Endothelial Growth Factor: Cytosol Levels in Primary Breast Cancer and Correlation to Established Prognostic Factors.

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Vascular endothelial growth factor (VEGF) is a potent angiogenic cytokine. The serum and plasma levels have been shown to have prognostic significance in patients with tumors of several origins. Most of the sudies used serum levels although it is known that the serum level of VEGF is influenced by platelet counts. Therefore in our study we analysed VEGF in the cytosol of primary breast cancer tumors. It was postulated that VEGF expression is different in lobular and ductal carcinomas.

Patients and methods: We studied retrospectively 173 cryopreserved samples of primary ductal and lobular breast cancer tissue from patients with stage I and II breast cancer obtained at primary therapy. 116 samples were ductal and 57 lobular carcinomas. The patients had a median age of 56 years, half of the patients were node negative (80/173), they underwent either radical or breast conserving surgery plus radiation therapy . Depending on nodal status they received chemotherapy and had a median follow- up of 28 months. The samples were homogenised by ultra-turrax. Cytosol levels of VEGF were measured using enzyme -linked immunosorbent assay (Quantikine, R&D systems, Oxon, UK)

Results: VEGF levels were significantly lower in the lobular carcinomas compared to ductal carcinomas. In invasive lobular carcinomas the median was 57 pg/mg total protein and in invasive ductal carcinomas it was 266 pg/mg total protein. As cut - off level served each median separately. Values above the median were regarded as positive and below as negative.

We found significant correlation of VEGF levels with grading, negative hormone receptor status and histopathology i.e. with markers of proliferation. We could not confirm a correlation with the nodal status and the number of lymph nodes involved.

Conclusion: In conclusion VEGF level in the cytosol of primary breast cancer tissue is a weak prognostic marker, but it was found to be an indicator for high proliferative tumors and might be used as selection criteria for antiangiogenic therapy.

567 Comparative Assessment of Lymphagenesis and Angiogenesis in Breast Carcinomas and Measurement of the Expression of Vascular Endothelial Growth Factor (VEGF-C, VEGF-A).

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Background: Very few studies have yet addressed the question of the role of lymphagenesis in tumor growth; it is generally overshadowed by the greater emphasis placed on the blood vascular system. Vascular endothelial growth factor C (VEGF-C) has been identified as a growth factor for the lymphatic system. It binds to the VEGFR3 tyrosine kinase receptor.

Design: The lymphagenesis of 60 breast carcinomas of 2cm, wathever their axillary lymph nodes status, was investigated by immunohistochemistry on frozen sections. Antibodies developped against VEGF-C and VEGFR3 (gifts from Dr. K. Alitalo or purchased from SantaCruz) provided a specific antigenic marker for lymphagenesis growth. A comparison with the microvascular count (MVC) immunostained on paraffin sections by the CD31 (Dako) and VEGF (SantaCruz) was done. VEGF-C and VEGF-A was quantified by image analysis (SAMBA)

Results: VEGFR3 staining was specifically localized in the endothelium of the lymphatic vessels allowing microlymphatic count (MLC) and VEGF on the tumoral cells . The mean value of MVC was 72.5 and that of MLC 40.5. The mean value of VEGF-A and VEGF-C was 5.3 and 4.4.

There was no correlation between these four parameters. The MVC, but not the MLC had a prognostic value in overall survival. Any was predictive of axillary lymph nodes invasion.

Conclusion: VEGF-C/VEGFR3 is a prominent system in lymphagenesis. The study of this process is in its infancy but may be of important value in breast cancer management.

568 Prognostic Significance of Angiogenesis Associated with Long-Term Survival in 377 Japanese Patients with Breast Cancer.

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Introduction: This study was undertaken to re-evaluate the value of angiogenesis and conventional prognostic factors in the predicting 20-year relapse-free survival (RFS) and 20-year overall survival (OS) rates in 377 Japanese patients with breast cancer. **Patients and Methods**: Three hundred seventy-seven patients with breast cancer, operated on during the period between 1971 and 1987, were studied. To evaluate the best objective method to quantify microvessel density in angiogenesis, average microvessel count (AMC) per square millimeter was employed (Kato et al, Breast Cancer Res Treat, 53, 1999). We investigated 5 factors, including angiogenesis, node status (n), clinical tumor size (T), histological grade (HG), and tumor necrosis (TN), followed for a median of 10 years. **Results**: Sixty-seven patients (17.8%) had recurrence and 54 patients (14.3%) died of breast cancer. Univariate analysis showed that n, T, HG, and AMC (P=.0020) were significantly predictive of RFS but that TN was not. n, T, HG, but not TN were significantly associated with OS but AMC was borderline significant (p=.0630). Multivariate analysis showed that n, T, HG, and AMC (p<.0001) were all significant and independent prognostic factors for RFS. n, T, HG, and AMC (p=.0034) were significantly associated with OS. When stratified by T, a significant impact of AMC on RFS or OS was seen both in patients with T2 and T3 carcinomas, but not in patients with T1 carcinomas. That of n and HG on RFS or OS was seen both in patients with T1 and T2 carcinomas, but not in patients with T3 carcinomas. When stratified by n, a significant impact of AMC on RFS or OS was seen in node-negative patients, but not in node-positive patients. That of T and HG on RFS or OS was seen both in node-negative and node-positive patients. **Conclusions**: We can confirm angiogenesis as a significant independent prognostic factor associated with long-term survival in Japanese breast cancer patients. It was especially considered a useful prognostic factor in node-negative patients and in patients with T2 and T3 carcinomas.

569 A Combination of NK4, a HGF/SF Variant and Gamma Linoleic Acid (GLA) Increases Inhibition of HGF/SF Stimulated Human Vascular Endothelial Cell Angiogenesis.

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Angiogenesis has been recognized as a prime requirement for tumour metastasis. NK4 is a newly discovered variant of HGF/SF (Hepatocyte Growth Factor/Scatter Factor) that has already been shown by us to be antagonistic to HGF/SF, a cytokine regulating cell motility, migration, proliferation, morphology and angiogenesis. GLA, a n-6 polyunsaturated fatty acid has previously been shown to inhibit HGF/SF stimulated tumour cell motility and invasion.

This study sought to examine the effect of NK4 and GLA in combination on (i) HGF/SF induced change in angiogenesis, (ii) HGF/SF induced change in endothelial cell migration. Angiogenesis was assessed using the human umbilical vein endothelial cell (HUVEC) tubule formation assay. Application of HGF/SF (25 ng/ml) alone caused an increase in tubule length (relative length 14.1±1.4 versus 3.8±2.6 in control). Addition of NK4 (400 ng/ml) and/or GLA (50 μM) inhibited this stimulation (HGF/SF with NK4 alone 6.2±0.6; with GLA; 7.9±1.7 with both 5.2±2.8; p=0.0008). Confluent layers of HUVEC were wounded to create a migration area. Application of HGF/SF (20 ng/ml) alone caused an increase in cell migration into the clear space (41.04±8.09μm versus 13.92±3.34 in control). Addition of NK4 (50 ng/ml) and/or GLA (50 μm) inhibited this stimulation (HGF/SF with NK4 alone 10.02±7.36; with GLA 18.59±3.72; with both 2.02±2.26; p=0.002).

In conclusion, we report that both the HGF/SF antagonist NK4 and GLA inhibited HGF/SF stimulated HUVEC migration and angiogenesis. Moreover, a combination of NK4 and GLA was more effective in preventing HGF/SF stimulation. NK4 and GLA may therefor have a role to play in controlling tumour angiogenesis.

570 Serum Deprivation Activates the Na+/H+ Exchanger and Invasion Via PKA-Dependent Phosphorylation of RhoA and Subsequent Down-Regulation of ROCK and p38 MAPK.

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One of the most characteristic phenotypes of cancer cells is their elevated resting intracellular pH (pHi) and acidic extracellular pH. The acidic extracellular pH of tumors plays a crucial role in extracellular matrix degradation while the alkaline pHi stimulates their movement, both processes being crucial for invasion. We have previously shown that low nutrient conditions, ie. serum deprivation, stimulates the Na+/H+-exchanger (NHE) in human breast cancer cells and also confers increased tumor motility and invasive ability that were abrogated by specific inhibition of the NHE. The regulation of this pathophysiological response of tumor cells to the tumor microenvironment is probably orchestrated by complex signal transduction systems. While much has been learned of the kinetics and action of these cascades in normal cells, very little is known about their actual function in neoplastic cells and their role in neoplastic processes and malignant progression. We studied the signaling module underlying the serum deprivation-dependent activation of the NHE in a cell line derived from a metastatic human breast tumor, MDA-MB-435. This stimulation of NHE activity by serum deprivation was potentiated by pharmacological inhibition of p38 MAP kinase and abrogated by inhibition of Protein Kinase A. Furthermore, inhibition of RhoA by C3 exotoxin or N19RhoA mimicked the effects of activation of PKA on NHE activity while activation of RhoA by the toxin CNF-1 or V14RhoA mimicked the inhibition of PKA on NHE activity. The same results were obtained with dominant negative and constitutively active mutants of the down-stream RhoA effector, p160ROCK. Dominant negative mutants of either RhoA or p160ROCK potentiated serum deprivation-dependent inhibition of p38 MAP kinase activity. Serum-deprivation increased the PKA-dependent phosphorylation of RhoA and transfection of the cells with a phosphorylation deficient mutant of RhoA (A188) abrogated this phosphorylation and the stimulation of NHE activity by serum deprivation. These results are consistant with a model in which serum deprivation-dependent activation of a PKA pathway inhibits the RhoA/p160ROCK/p38 pathway. Suppression of this inhibitory pathway releases the cells from its constraint and enables the activation of the target process, NHE activity. An autocrine loop driven by ATP-dependent changes in the cytoskeleton might underly this phenomenon.

571 Interaction of the Differentially Expressed S100A7 Gene with Centrosomal Proteins.

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We previously utilized a subtraction hybridization technique to identify the S100A7 (psoriasin) gene as being differentially expressed between different stages of human breast cancer. Originally discovered as being highly expressed in abnormally differentiated squamous keratinocytes in patients with psoriatic lesions, S100A7 was observed to be a secreted protein with chemotactic ability for $T4^+$ lymphocytes. We recently used In-situ hybridization, RT-PCR, IHC and Western blot to confirm high S100A7 expression in DCIS compared to normal and invasive breast tissue. Protein expression was also found to be present in both the nucleus and the cytoplasm. However, maintance of protein expression in breast tumors was found to correlate with ER(-) status and poor prognosis.

S100A7 is a member of the S100 family of proteins. Members of this family share numerous similarities such as containing Ca^{2+} binding EF-hand domains, location in a gene cluster at position 1q21 and a molecular mass of about 10 KDa. Currently, there are no data on the biological role of S100A7, and how its altered expression contributes to the biological process of invasion. To address this question and identify potential pathways or cellular processes in which S100A7 participates, we utilized the yeast two-hybrid assay to identify interacting proteins. Full length S100A7 was used to screen $17.3x10^6$ clones from a normal human mammary cDNA library. Two centrosomal proteins, RanBPM and hGCP3, were determined to be true positives in the yeast assay and were selected for further study. As well, confocal microscopy analyses show that S100A7 does indeed localize to the centrosome. The biological importance of these interactions is under investigarion.

Ethan Emberley is the recipient of a USAMRMC Predoctoral Traineeship Award.

572 The RXR-Selective Retinoid LGD1069 Inhibits Breast Cell Growth through Cell Cycle Blockade and Activation of Other Receptor Pathways.

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Retinoids are being investigated for the treatment and prevention of cancer. While naturally-occurring retinoids have been shown to suppress cancer, their toxicity has prevented their widespread use. Recently, receptor-selective retinoids have been developed which suppress carcinogenesis without these toxic side effects. Our laboratory has previously shown that retinoids selective for the retinoid X receptor (RXR) inhibit the growth of normal and malignant breast cells *in vitro*. In addition, we have demonstrated that RXR-selective retinoids are able to suppress tumor development in animal models of breast cancer. In the present study, we have examined the mechanisms by which these agents suppress cancer. Analysis of cell cycle using flow cytometry and tritiated thymidine incorporation analysis demonstrated that LGD1069 induces a G1 cell cycle block in T47D breast cancer cells. Using TUNEL assays, we observed that this retinoid does not induce apoptosis in MCF7 breast cancer cells, but does induce apoptosis in T47D breast cancer cells. Some of the effects of the RXR-selective retinoid may be through interactions with dimerization partners including the retinoic acid receptor (RAR), the peroxisome proliferator-activated receptor (PPAR), and the vitamin D receptor (VDR). To investigate whether RAR-activation is required for RXR-ligands to inhibit breast cell growth, we attempted to block the antiproliferative effect of RXR-selective ligands with a specific RAR-antagonist LGD100815. These results showed that growth inhibition by the RXR-selective retinoid LGD1069 was not relieved by the addition of LGD100815. We have also investigated the ability of these agents to activate these RAR-, PPAR-, and VDR-dependent pathways in both normal and malignant breast cells. These results demonstrate that RXR-selective retinoids are able to strongly activate reporter genes containing RXR- and PPAR-responsive elements in breast cells. Examination of endogenous genes in breast cell lines treated with retinoids further shows induction of these pathways. The ability of these retinoids to suppress carcinogenesis is likely mediated by a combination of these mechanisms. Studies are under way to determine the pathways associated with chemoprevention by these compounds.

573 Antitumor Action of Estradiol on Estrogen-Deprived or Raloxifene-Resistant Human Breast Cancer Cells.

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We have recently reported that physiological dose of estradiol (E_2) has inhibitory effect on the growth of 5 year tamoxifen (TAM)-stimulated tumors in mice (Yao K, et al. Clin Cancer Res 2000;6(5): 2028-36). To understand the antitumor effect of E_2, we have developed MCF-7/ED and MCF-7/Ral cells by growing MCF-7 cells in estrogen-deprived or in 10^{-6} M raloxifene-containing medium for more than 12 months. Both derived cell lines had enhanced ERa levels measured by western blot analysis. The results from transient transfection assay using a vit-ERE3 luciferase reporter showed that ERa in MCF-7/ED or MCF-7/Ral cells was functional. However, MCF-7/ED and MCF-7/Ral cells had distinctive mophologies from parental MCF-7 cells. E_2 was less stimulatory in MCF-7/Ral cells and not stimulatory at all in MCF-7/ED cells. Instead, E_2 was inhibitory on cell growth at higher concentration (10^{-10} to 10^{-8} M) in MCF-7/ED. After inoculating into athymic mice, untreated MCF-7/ED cells grew faster than the E2 group during the first 9 weeks. Interestingly, tumor sizes in the no treatment group were significantly decreased after inplanting E_2 capsule. MCF-7/Ral cells did not grow in the absence or presence of E_2. However, they were stimulated to grow by raloxifene or tamoxifene. Tumors also regressed after giving E_2 as observed previously for MCF-7/ED cells in vivo. Overall, the results from the current and previous studies suggest that breast cancer cells become supersensitive to E_2 after long-term estrogen deprived condition, so that E_2 inhibits their growth in vivo.

Supported by the Avon Breast Cancer Fund.

574 The Role of Differential Expression of Extracellular Matrix Proteins Regarding Chemoresistance of Breast Cancer Tissue in Nude Mice.

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Introduction: Resistance to chemotherapy is a principal problem in the treatment of breast cancer. It is known that both primary tumors and metastastic sites of human breast cancer are extensively surrounded by stroma of extracellular matrix (ECM). Components of the ECM seem to play an important role in cancer growth and the development of chemoresistance.

Methods: Native and chemoresistant cell lines of human breast cancer (native: mcf-7, resistant: mcf-7R-NOV, resistant to novantrone) were injected intraperitoneally into nude mice (n=160). In eighty animals, prior to tumor cell injection, a CO2-pneumoperitoneum with an intraperitoneal pressure of 6 mmHg was applied over 30 min in order to enhance tumor cell adhesion to peritoneal surface and to induce an increased production of ECM. Five days after the injection 40 animals of each group were treated with Novantrone i.p.. Animals were sacrificed after 6, 12, 24, 48 and 72 hours. Tumor tissues and parts of the parietal peritoneum were formalin-fixed and embedded into paraffin. Immunhistochemic examination of the tissue was done with antibodies against laminin (LAM-89), fibronectin (FN-3E2), tenascin (BC-24) and collagen IV (Col-94).

Results: The greatest volume of tumor mass was found in those nude mice that underwent CO2-pneumoperitoneum and i.p.-injection of mcf-7R-NOV. The lowest volume of tumor mass was seen in nude mice not treated with CO2-pneumoperitoneum and exponed to native mcf-7.

An increase in ECM in tumor tissue in comparison to normal peritoneum was a consistant finding. Furthermore, significant qualitative and quantitative differences between the ECM-components of chemosensitive and chemoresistant tumor tissue as well as CO2 treated and untreated animals could be revealed.

Conclusion: By means of this animal model it is possible to induce the production of peritoneal ECM. Thus enables us to examine the influence of ECM on the development of chemoresistance of human breast cancer cells in vivo. The above data suggest that there is a creation of a specialized environment as a consequnce of autocrine and paracrine effects that induces increased tumor growth and reduces the cytotoxicity of chemotherapeutic agents.

575 Decreased Response to Paclitaxel Versus Docetaxel in a HER-2/neu Transfected Cell Line.

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Paclitaxel and docetaxel are antineoplastic agents belonging to the taxane family. It has been reported that overexpression of HER-2/neu may confer resistance to paclitaxel in human breast cancer cells (Yu, et al. Oncogene 13 [6]: 1359-65, 1996). Docetaxel induces bcl-2 phosphorylation and subsequent apoptosis at 100-fold lower concentrations than paclitaxel and is clinically active in patients with paclitaxel-resistant breast cancer. The purpose of this study was to compare docetaxel and paclitaxel activity in a HER-2/neu transfected breast cancer cell line.

Using a human breast cancer cell line with minimal HER-2/neu expression (MCF/neo) and its corresponding HER-2/neu transfected line (MCF/18), we compared the effects of docetaxel and paclitaxel (0.5-5 nM) on cell growth. Docetaxel inhibited cell growth in the same dose-dependent manner in both cell lines (2.5 nM: 44% inhibition). Paclitaxel produced similar results in the MCF/neo breast cancer line (2.5 nM: 31% inhibition). The response to paclitaxel, however, was significantly reduced in the HER-2/neu transfected MCF/18 line (2.5 nM: 17% inhibition).

In summary, the HER-2/neu transfected breast cancer cell line, MCF/18, was more sensitive to docetaxel than paclitaxel. These results suggest that docetaxel may be the preferred taxane therapy in HER-2/neu positive breast cancer.

576 Molecular Classification of High Risk Breast Cancer Patients by Gene Expression Profiling.

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For many tumors pathological subclasses exist which have to be further defined by genetic markers to improve therapy and follow up strategies. Recently, the development of the array hybridization techniques based on cDNA or oligonucleotides enabled the parallel expression profiling of several thousand genes, providing a powerful tool for characterizing complex cellular transcriptional activities. These DNA-arrays have been used to identify gene expression patterns in various types of cancer tissues and to elucidate signalling pathways. At present one major aim is to use DNA arrays as a tool to understand and classify tumors into categories based on shared gene expression patterns. It is anticipated that global determination of cellular transcriptional activity will identify gene expression signatures that predict clinical behaviour of tumors.

We have performed several low and high density cDNA array expression analyses to identify transcriptional changes among human breast cancers. Genes, repeatedly found to be differentially expressed, were subsequently applied to perform a molecular tumor classification of normal and malignant breast specimens by cluster analysis. Here we show that this class discovery analysis identifies two larger tumor subgroups, class A (further subdivided in A1 / A2) and class B. Cross validation analysis of the samples yields prediction strengths significantly higher than would be expected for random class distinctions. Correlation of cluster data with classical clinicopathological parameters revealed, that subgroup A1 was characterized by a remarkable high number of nodal positive tumors. Strikingly, in this subgroup we also observed an accumulation of samples from patients which had already developed distant metastases at the time of diagnosis. The total percentage of these M1 patients was determined to be 25 % in this subgroup compared to 4 % among the rest of the malignant samples. We observed no correlation between cluster data and tumor size, grading or histological subtype. Taken together, these cluster analysis data may contribute to define high risk breast cancer patients with an early onset of disease progression, as a first step towards improved patient adapted therapy.

577 Microarray Analyses of Gene Expression Regulated by ErbB-2: Interactions with Estrogen Receptor (ER) Activation/ Inhibition.

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Amplification/overexpression of the ErbB-2 (HER2/neu) proto-oncogene has prognostic and predictive value in breast cancer patients. Therapeutic strategies targeting ErbB-2 have proven to be efficacious for some, but not all, breast cancer patients with ErbB-2 overexpressing/amplified tumors. Differences that account for this variable responsivity have yet to be defined. Interactions between ErbB-2 and hormonal pathways have also been suggested by clinical trials, which have demonstrated tamoxifen resistance in ER+, EGFR/ErbB-2 abnormal breast cancer patients. Therefore, analyses of gene expression patterns/pathways associated with ErbB-2 and hormone signaling pathways are of great interest. Gene expression profiles of the MCF-7 and MCF-7 transfected with ErbB-2 (with estrogen, +/- tamoxifen) were analyzed using microarray technology. Control and ErbB-2 overexpressing MCF-7 cell lines were starved in serum and phenol red free medium supplemented with 1% BSA for 72 hours, then stimulated with E2 for 10 hours. RNA samples from each cell line were prepared from starved cells and E2 stimulated cells. ER dependent gene expression was also compared to control and ErbB-2 overexpressing MCF-7 cell lines treated with hydroxytamoxifen for 12 hours. Gene profiles were also generated from SK-BR3 cells with naturally amplified ErbB-2 treated with Herceptin, a therapeutic antibody targeting ErbB-2. Differential gene expression by these breast cancer cell lines, with and without hormonal or ErbB-2 blockade, will be presented. Supported by the Carol Gollob Foundation.

578 Prospective Study of Signal Transduction Pathways Associated with Response and Resistance to Herceptin®-Based Therapy for Patients with Metastatic Breast Cancer.

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HER-2 is amplified or overexpressed in 25-30 percent of invasive breast carcinomas and its overexpression has been associated with a poor prognosis. Data suggest an association between HER-2 overexpression and resistance to chemotherapy. Herceptin is a humanized monoclonal antibody that binds Her-2 causing growth arrest of HER-2 overexpressing cancer cells. Experimental data shows synergism between Herceptin and various chemotherapeutics that results in prolonged time to disease progression and improved response rates for patients with metastatic breast cancer overexpressing HER-2. However, a significant number of patients whose tumors overexpress HER-2 do not respond to Herceptin.

Recently, the ability of trophic factors to promote survival has been attributed, at least in part, to the phosphatidylinositide 3'-OH kinase (PI3K)C-Akt kinase cascade. For example, a member of the Akt family, Akt2, plays an important role in cell survival and in blocking programmed cell death or apoptosis after radiation therapy or chemotherapy.

This pilot study was aimed to understand the molecular mechanisms associated with response and resistance to Herceptin in patients who were to be treated with Herceptin. Ten patients underwent needle biopsies of an amenable metastatic lesion before and after initiation of Herceptin. The tumor samples were frozen in liquid nitrogen and processed for immunohistochemistry, RT-PCR and western blot analysis. Levels of EGFR, HER-2, HER-3, HER-4, Heregulin, and phosphorylated HER-2, Akt1, Akt2, ERKs and MAPKp38 were tested. Our results show that upregulation of various genes associated with survival, mainly in the PI3 kinase pathway, may be implicated in response to Herceptin. This study is likely to contribute to the development of tests predicting response to Herceptin, both as a single agent and in combination with chemotherapy.

579 Somatic Genetic Alterations in Inflammatory Breast Cancer (IBC).

Lerebours F,[1,2] Bièche I,[1,3] Bertheau P,[2] Turpin E,[2] Plassat F,[2] Vidaud M,[3] De Roquancourt A,[2] Janin A,[2] De Thé H,[2] Lidereau R.[1] [1]Centre René Huguenin, Saint-Cloud; [2]Hôpital Saint-Louis, Paris; [3]Paris V, France.

IBC (Inflammatory Breast Cancer) only represent 1 to 2% of all diagnosed breast cancers, and is of very unfavorable prognosis. Tumor-biological factors have been poorly studied in IBC. Moreover, IBC has been rarely distinguished from LABC (Locally Advanced Breast Cancer), despite different prognostic profiles. We hypothesize that distinct genetic features between IBC and non-IBC might explain their prognostic differences. We investigated a series of 47 IBC diagnosed at Saint-Louis Hospital between 1988 and 1999. In this series, we screened the most frequent genetic alterations that have been described in non-inflammatory breast cancer: gene alterations of TP53, ERBB2, MYC, CCND1, and LOH at 1p, 3p, 6q, 8p, 9p, 11q, 13q, and 16q using 60 polymorphic markers. Expression of ERBB2, MYC and CCND1 were performed by quantitative real-time PCR and TP53 mutations by FASAY assay. A high frequency of TP53 mutations (61% : 27/44), ERBB2 overexpression (40%), and LOH at 8p, 11q and 16q (≥ 40%) were found. Further genetic characterization of this series and of additional IBCs is in progress. Since clinical and pathological data are available, we also investigate correlations between molecular data and response to chemotherapy, DFS and OS.

580 A Rapid and Cost-Effective Method for Detecting Gene Mutations in Breast Carcinomas: Screening for TP53 Sequence Variants Using Denaturing High Performance Liquid Chromatography (DHPLC) on the WAVE™ Platform.

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Mutations of the p53 tumor suppressor gene (TP53) appear to be the most common genetic alteration found in human cancers. TP53 mutations in breast cancers have been screened for extensively using costly and labor-intensive methods such as single-stranded conformational polymorphism (SSCP) analysis, immuno-histochemistry, and direct DNA sequencing of the TP53 gene. We have a developed a simple and reliable method for the identification of p53 gene mutations in cancer tissues using PCR and DHPLC analysis on the WAVE™. TP53 exons 4 through 9 are amplified from genomic DNA using PCR with intron-specific primers optimized for DHPLC. After amplification, the PCR products are directly analyzed for TP53 mutations by DHPLC. A single sample can be analyzed for mutations in 5 to 7 minutes and 200 samples can be individually screened in less than a day. With pooling of 4 to 5 samples, approximately 800-1000 samples can be analyzed in 24 hours. Any variants detected by DHPLC can be confirmed by direct sequencing of the appropriate eluted DNA fragments with the platform fraction collector. We present application data for DHPLC screening of breast tissues for TP53 gene mutations. This approach is rapid, automated, and reduces the costs of mutation screening dramatically.

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